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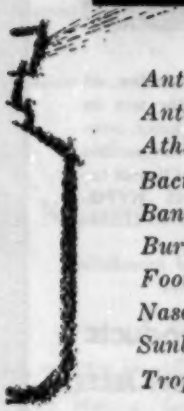

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

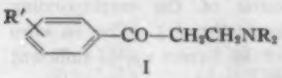
VOLUME XLVII | *FEBRUARY 1958* | NUMBER 2

Ketonic Mannich Bases and the Products of their Reduction and Bromination*

By W. LEWIS NOBLES† and J. H. BURCKHALTER

A group of ketonic bases, indicated as types I and II, were synthesized by means of the Mannich reaction for pharmacological study. Certain of the type I Mannich bases were then reduced by means of aluminum isopropoxide to nitrophenyl amino alcohols (type III) for use as side chains in future studies. Other type I bases were brominated to give ketonic nitrogen mustards for study against cancer. Except for appreciable *in vitro* antituberculous activity of some of the Mannich bases, there were no noteworthy pharmacological results.

INTEREST HAS BEEN EXPRESSED in the pharmacological activity of various ketonic Mannich bases (1). Therefore, we wished to prepare a number of unavailable Mannich bases of types I and II and to submit them for pharmacological



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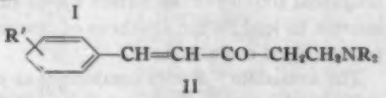
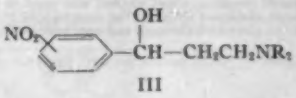


II

testing. Another important planned phase of these studies involved the reduction of type I, where R′ is nitro, to compounds of type III which would be employed as inter mediates in the synthesis of potential amebacides, as described in the next paper which appears in THIS JOURNAL. Further, it was planned to obtain a group of ketonic nitrogen mustards of type IV as possible anticancer agents.



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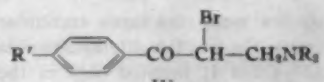


IV

The preparation of compounds of types I and II, which are listed in Tables I and II, respectively, was carried out by means of the Mannich reaction (2). It involved the interaction of formaldehyde or paraformaldehyde with the appropriate ketone and amine hydrochloride, illustrated as follows:

$$C_6H_5COCH_3 + CH_2O + HNR_2 \cdot HCl \rightarrow C_6H_5COCH_2CH_2NR_2 \cdot HCl + H_2O$$

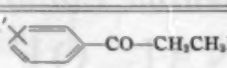
In general, the reactions proceeded smoothly and in good yields to give the expected product. The fact that excellent yields are seldom obtained in the Mannich reaction involving ketones is understandable when it is noted that there is more than one replaceable hydrogen in the ketone ordinarily employed, which leads to by-product formation. Also, inconsistent results in our

* Received July 26, 1957, from the Laboratory of Pharmaceutical Chemistry, University of Kansas School of Pharmacy, Lawrence, Kan.

Extracted from a portion of the Ph. D. thesis of W. Lewis Nobles, 1952. For the first paper extracted from this thesis, see THIS JOURNAL, 42, 176(1953).

† Fellow of the American Foundation of Pharmaceutical Education. Present address: School of Pharmacy, University of Mississippi, University, Miss.

TABLE I.—β-DIALKYLAMINOPROPIOPHENONES



No.	R′	NR_2	Yield, %	M. P., °C.	Formula	Ionic Calcd.	Halogen Found
1	*p*-Chloro	Dimethylamino	71	176	$C_{11}H_{14}ClNO \cdot HCl$[a,b]	...	...
2	*p*-Chloro	Diethylamino	60	145	$C_{13}H_{18}ClNO \cdot HCl$[a]	12.84	12.83
3	*p*-Chloro	1-Piperidyl	56	190	$C_{14}H_{18}ClNO \cdot HCl$[c]	12.30	12.37
4	*p*-Bromo	Dimethylamino	52	196	$C_{11}H_{14}BrNO \cdot HCl$[d]	...	...
5	*p*-Bromo	Diethylamino	40	166	$C_{13}H_{18}BrNO \cdot HBr$	21.88	22.01
6	*p*-Bromo	1-Piperidyl	42	188	$C_{14}H_{18}BrNO \cdot HBr$	21.19	21.12
7	*p*-Bromo	1-Pyrrolidyl	69	199	$C_{13}H_{16}BrNO \cdot HCl$	11.13	11.29
8	*p*-Methoxy	Dimethylamino	75	181	$C_{12}H_{17}NO_2 \cdot HCl$[e]	...	...
9	*p*-Methoxy	1-Pyrrolidyl	38	184	$C_{14}H_{19}NO_2 \cdot HCl$	13.14	13.22
10	*p*-Methoxy	Benzylamino	29	183	$C_{17}H_{19}NO_2 \cdot HCl$[f]	...	...
11	*m*-Methoxy[g]	Dimethylamino	81	168	$C_{12}H_{17}NO_2 \cdot HCl$[d]	...	...
12	*o*-Hydroxy	Dimethylamino	33	156	$C_{11}H_{15}NO_2 \cdot HCl$	15.43	15.55
13	*m*-Hydroxy[i]	Dimethylamino	50	180	$C_{11}H_{15}NO_2 \cdot HCl$[d]	...	...
14	*p*-Hydroxy[h]	Dimethylamino	56	192	$C_{11}H_{15}NO_2 \cdot HCl$[d]	...	...
15	*p*-Phenyl[j]	Dimethylamino	69	192	$C_{17}H_{19}NO \cdot HCl$	12.24	12.39
16	*p*-Nitro[m]	Dimethylamino	72	191	$C_{11}H_{14}N_2O_3 \cdot HCl$[g]	13.71	13.80
17	*p*-Nitro	Diethylamino	66	150	$C_{13}H_{18}N_2O_3 \cdot HCl$[k]	12.36	12.22
18	*p*-Nitro	1-Pyrrolidyl	61	185	$C_{13}H_{16}N_2O_3 \cdot HCl$[k]	12.45	12.60
19	*p*-Nitro	1-Piperidyl	51	200	$C_{14}H_{18}N_2O_3 \cdot HCl$[k]	...	...
20	*p*-Nitro	Dipropylamino	26	140	$C_{15}H_{22}N_2O_3 \cdot HCl$	11.26	11.39
21	*p*-Nitro	Diethanolamino	19	146	$C_{13}H_{18}N_2O_5 \cdot HCl$[k]	11.12	11.31
22	*p*-Nitro	4-Morpholinyl	62	218	$C_{13}H_{16}N_2O_4 \cdot HCl$[k]	11.79	11.82
23	*m*-Nitro[n]	Dimethylamino	72	205	$C_{11}H_{14}N_2O_3 \cdot HCl$[c]	...	...
24	*m*-Nitro	Diethylamino	..	122	$C_{13}H_{18}N_2O_3 \cdot HCl$[c]	...	...
25	*m*-Nitro	1-Pyrrolidyl	63	182	$C_{13}H_{16}N_2O_3 \cdot HCl$	12.45	12.54
26	*m*-Nitro	1-Piperidyl	..	180	$C_{14}H_{18}N_2O_3 \cdot HCl$[c]	...	...

[a] Adamson, D. W., and Billinghurst, J. W., *J. Chem. Soc.*, 1950, 1039.
[b] Dhont, J., and Wibaut, J. P., *Rec. trav. chim.*, **63**, 81(1944).
[c] Denton, J. J., *et al.*, *J. Am. Chem. Soc.*, **71**, 2048(1949).
[d] Knott, E. B., *J. Chem. Soc.*, **1947**, 1190.
[e] Mannich, C., and Lammering, D., *Ber.*, **55**, 3510(1922).
[f] Prepared by John A. Durden, This Laboratory. *Anal.* Calcd. C, 66.76; H, 6.59. Found: C, 66.74; H, 6.38.
[g] See ref. 5.
[h] See ref. 5-c.
[i] Mannich, C., and Dannehl, M., *Arch. Pharm.*, **276**, 206(1938).
[j] Intermediate *m*-methoxyacetophenone made from Hilton-Davis *m*-hydroxyacetophenone, according to Auwers, K. V., *et al.*, *Ann.*, **408**, 212(1915).
[k] *p*-Hydroxyacetophenone obtained through the courtesy of Dow Chemical Co.
[l] To effect the Mannich reaction, amyl alcohol was used as the solvent.
[m] *p*-Nitroacetophenone obtained through the courtesy of Monsanto Chemical Co.
[n] *m*-Nitroacetophenone obtained through the courtesy of Carbide and Carbon Chemicals Corp.

studies were sometimes encountered. For example, the first two attempts to obtain compound 15 (Table I) resulted only in the recovery of starting material. However, when the customary solvent alcohol was replaced by isoamyl alcohol, uniformly good yields were obtained. The latter alcohol, having a higher reflux temperature, presumably afforded the necessary elevation of temperature to effect reaction. Under the conditions employed, it was not possible to cause 3,5-dinitroacetophenone or *p*-nitro-α-acetamidoacetophenone, a chloramphenicol intermediate, to undergo the Mannich reaction, although the latter will easily hydroxymethylate (3).

The *p*-nitro-β-dialkylaminopropiophenones of Table I are so unstable that they decompose readily in the presence of dilute alkali. However, they can be prepared and stored for some time in the form of their hydrochloride salts. The ready decomposition of ketonic Mannich bases into α,β-unsaturated ketones and amines is well established, although they generally are not so unstable as the ones under discussion (4).

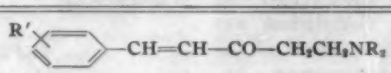
A Mannich base from *p*-nitroacetophenone (Compound 16, Table I) apparently was first prepared in This Laboratory (5), and it was made as the parent compound of the corresponding vinylog referred to earlier (1). The *in vitro* fungicidal activity of the former was of sufficient interest to lead to the synthesis of several compounds listed in Table I.

The availability of such compounds as *p*-nitro-β-dimethylaminopropiophenone (5) (Table I) suggested them as possible intermediates to be used as side chains in the synthesis of products of interest as amebacidal and antimalarial agents. In This Laboratory attempts were made by Wei-Ling to reduce such compounds to the corresponding 1-(*p*-aminophenyl)-3-dialkylaminopropanols or ketones (6). While the catalytic reduction of *m*-nitro-β-piperidylpropiophenone hydrochloride to the corresponding *m*-aminophenyl

ketone has been reported (7), the melting point and solubility of the product were found to change rapidly upon standing (6). The logical explanation of the instability of the desired compounds is the formation of Schiff bases, and thus it appeared that a means must be found of reducing type I compounds to the stable nitro amino-alcohols (III), which later could be reduced further catalytically to the desired side-chain amines.

It was evident that, owing to their instability, a successful reduction of the type of Mannich base in question (I) must be applied directly to the amine salt. The Meerwein-Ponndorf-Verley method, employing aluminum isopropoxide, appeared to fulfill the requirements, for, in contrast to other reductions involving metals in acid or alkaline media or even certain catalytic hydrogenations, carbon-carbon double bonds, nitro

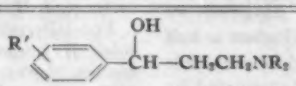
TABLE II.—δ-DIALKYLAMINO-1-PHENYL-1-PENTEN-3-ONES



No.	Phenyl Substituents	NR_2	Yield, %	M. P., °C.	Formula	Chlorine Calcd.	Chlorine Found
1	None[a]	4-Morpholinyl	66	178	$C_{15}H_{19}NO_2 \cdot HCl$	12.58	12.46
2	None[a]	1-Pyrrolidyl	42	178	$C_{15}H_{19}NO \cdot HCl$	13.34	13.51
3	2,3-Dimethoxy[b]	1-Pyrrolidyl	65	155	$C_{17}H_{23}NO_3 \cdot HCl$	10.88	11.01
4	p-Nitro[b]	1-Pyrrolidyl	57	196	$C_{15}H_{18}N_2O_3 \cdot HCl$	11.41	11.53

[a] Made from Eastman benzalacetone.
[b] For intermediate ketone, see ref. 1.

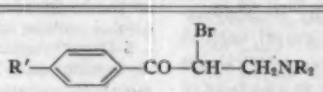
TABLE III.—3-DIALKYLAMINO-1-PHENYL-1-PROPANOLS



No.	R,	NR_2	Yield, %	M. P., °C.	Formula	Chlorine Calcd.	Chlorine Found
1	None	Dimethylamino	65	134	$C_{11}H_{17}NO \cdot HCl$[a]	...	...
2	p-Nitro	Dimethylamino	65	176	$C_{11}H_{16}N_2O_3 \cdot HCl$[b]	...	...
3	p-Nitro	Dimethylamino	54	140	$C_{12}H_{20}N_2O_3 \cdot HCl$	12.28	12.41
4	p-Nitro	1-Pyrrolidyl	61	168	$C_{13}H_{18}N_2O_4 \cdot HCl$	12.37	12.52
5	p-Nitro	1-Piperidyl	55	177	$C_{14}H_{20}N_2O_3 \cdot HCl$	11.78	11.91
6	p-Nitro	4-Morpholinyl	67	185	$C_{13}H_{18}N_2O_4 \cdot HCl$	11.71	11.66
7	m-Nitro	Dimethylamino	42	188	$C_{11}H_{16}N_2O_3 \cdot HCl$	13.59	13.71

[a] Mannich, C., and Heilner, G., *Ber.*, **55**, 356(1922) made by catalytic hydrogenation using palladium. See also Experimental section of this report.
[b] *Anal.*: Calcd. C, 50.67; H, 6.57. Found: C, 50.89; H, 6.47.

TABLE IV.—α-BROMO-β-DIALKYLAMINOPROPIOPHENONES



No.	R'	NR_2	Yield, %	M. P., °C.	Formula	Total ionic halide[d] Calcd.	Total ionic halide[d] Found
1	H	1-Piperidyl	85	185	$C_{14}H_{18}BrNO \cdot HBr$[e]	...	...
2	H[h]	4-Morpholinyl	85	181	$C_{13}H_{16}BrNO_2 \cdot HBr$	42.16	42.03
3	Cl[c]	Dimethylamino	78	191	$C_{11}H_{13}BrClNO \cdot HCl$[d]	35.27	35.39
4	Cl[g]	1-Piperidyl	82	175	$C_{14}H_{17}BrClNO \cdot HBr$	38.84	39.01
5	Br[f]	Diethylamino	80	150	$C_{13}H_{17}Br_2NO \cdot HBr$	35.99	35.87
6	Br[g]	1-Piperidyl	81	168	$C_{14}H_{17}Br_2NO \cdot HBr$	35.07	34.86
7	OCH_3[h]	Dimethylamino	86	169	$C_{12}H_{16}BrNO_2 \cdot HBr$	43.54	43.39
8	OCH_3[i]	1-Piperidyl	87	145	$C_{15}H_{20}BrNO_2 \cdot HBr$	39.25	39.30
9	NO_2[j]	1-Piperidyl	84	182	$C_{14}H_{17}BrN_2O_3 \cdot HBr$	37.87	37.88

[a] Ref. 10.
[b] Intermediate Mannich base hydrobromide made from morpholine hydrobromide in 52% yield, m. p. 192°. Not analyzed.
[c] See Table I, compound 1, for intermediate Mannich base hydrochloride.
[d] Note that the α-bromo group reacts with silver nitrate in the analytical procedure, and that the analysis of compound 3 includes both bromide and chloride ions.
[e] Intermediate Mannich base hydrobromide made in 55% yield, m. p. 205°. *Anal.*: Calcd.: Br, 24.02. Found: 23.78.
[f] Intermediate Mannich base hydrobromide, Table I, compound 5.
[g] See Table I, compound 6 for intermediate.
[h] Intermediate Mannich base hydrobromide made in 75% yield, m. p. 182°.
[i] Intermediate Mannich base hydrobromide made in 75% yield by John A. Durden, m. p. 223°. *Anal.* Calcd.: C, 54.88; H, 6.76. Found: C, 55.02.; H, 6.59.
[j] Intermediate Mannich base hydrobromide made in 50% yield, m. p. 189°.

groups and carboxylic esters are not reduced by this agent. This procedure had previously been applied by Lutz and associates (8) to Mannich bases derived from *p*-haloacetophenones, as well as to a number of α-amino ketones. While the reduction of the latter proceeded very smoothly, in only one case was the amino alcohol obtained, and the yield was less than 10%. These poor results were attributed to reductive fission of the dialkylamino groups (8). In the early stages of our studies, the same results were noted, for, in each case as the temperature was elevated, the evolution of the low boiling dialkylamine was detected (9). Meanwhile, the contents of the reaction vessel became dar'; and resinous. Apparently, as the amine was eliminated, polymerization of the residual phenyl vinyl ketone occurred.

The addition of aluminum chloride has been successfully employed by Lutz (8) to improve yields in the reduction of α-amino ketones by aluminum isopropoxide, although it apparently has not been used in the reduction of Mannich bases, which are β-amino ketones. However, we found this procedure to work well when a half mole of aluminum chloride was used for each mole of Mannich base. The compounds prepared by this method are outlined in Table III and are derived from those of Table I. However, the general procedure is illustrated in the Experimental section by application to a type II compound, which is vinylogous with type I.

A group of ketonic nitrogen mustard compounds of type IV (see Table IV) were synthesized by bromination of ketonic Mannich bases (type I) using the procedure of Land, Ziegler, and Sprague (10).

Pharmacological Results.[1]—The compounds of Table I were screened generally for antibacterial and antiamebic activity. Several, such as numbers 8, 10, and 22 showed interesting *in vitro* antituberculous activity but later *in vivo* tests in mice were negative. Others, such as 1, 4, 8, 16, and 21 showed promise against histoplasmosis but again were inactive *in vivo*. Compound 1, Table II, showed *in vitro* antituberculous activity, but failed in mice. Also, its antiamebic and analgetic activity were of too low an order to be of further interest.

The products of Table III were utilized as chemical intermediates and were not tested pharmacologically, while the ketonic nitrogen mustards of Table IV were found by Dr. Chester C. Stock of Sloan-Kettering Institute to be devoid of anticancer activity. This lack of activity is not surprising in view of the fact that known anticancer agents contain two β-haloethyl groupings, whereas ours have only one. Compound 8 did, however, show appreciable analgetic activity but was toxic to guinea pigs.

It is of passing interest to point out that the compounds of Table IV are strongly sternutatory. Their structural relationship to phenacyl chloride, an α-halo ketone and a famous tear gas, is noteworthy.

EXPERIMENTAL[2]

β-Dialkylaminopropiophenones (Table I).—The procedure of Maxwell (11) was employed for the synthesis of the ketonic Mannich base hydrochlorides of Table I. In general, 95% alcohol was used as the recrystallizing solvent, although compounds 1 and 2 were recrystallized from isopropyl alcohol while 3, 7, 9, 12, and 17 were purified by means of an alcohol-acetone mixture.

Most of the intermediate ketones were Eastman chemicals, otherwise the source is indicated in the footnotes to Table I.

2-(β-1-Pyrrolidylpropionyl)-thiophene Hydrochloride.—By means of the foregoing procedure, this compound was prepared in 48% yield, m. p. 157–162°. After recrystallization from alcohol-acetone, the m. p. became 169–170°.

Anal. Calcd. for $C_{11}H_{15}NOS \cdot HCl$: Cl, 14.43. Found: Cl, 14.60.

2-(β-Benzylaminopropionyl)-thiophene Hydrochloride.[3]—By means of the foregoing procedure, this compound was prepared in 25% yield, m. p. 172–173°. After recrystallization from absolute alcohol, the m. p. became 174–175°.

Anal. Calcd. for $C_{14}H_{15}NOS \cdot HCl$: C, 59.66; H, 5.72. Found: C, 59.36; H, 5.44.

5-Dialkylamino-1-phenyl-1-penten-3-ones (Table II).—The compounds of this table were prepared by the procedure of Table I. The products, obtained as the hydrochlorides, were recrystallized from an alcohol-acetone mixture.

5-Dimethylamino-1-(*p*-nitrophenyl)-1-penten-3-ol Hydrochloride.—To a hot slurry of 20 Gm. (0.1 mole) of aluminum isopropoxide and 3.3 Gm. (0.025 mole) of anhydrous aluminum chloride in 175 ml. of dry isopropyl alcohol contained in a 500-ml. three-necked flask equipped with a mechanical stirrer and reflux condenser, there was added 14.3 Gm. (0.05 mole) of 5-dimethylamino-1-(*p*-nitrophenyl)-1-penten-3-one hydrochloride (1). The mixture was brought to full reflux and maintained at that temperature for fifteen minutes. Then, the upright condenser was replaced by one turned downward for distillation, and stirring and removal of acetone continued until the distillate gave a negative acetone test (about two hours). The upright condenser was reinserted and full reflux maintained for about ten minutes. Once again, the upright condenser was replaced by one placed so as to allow

[1] The authors wish to thank Parke, Davis and Company for arranging for the pharmacological testing.

[2] Melting points are uncorrected.

[3] Prepared by John A. Durden of This Laboratory.

distillation to proceed and a few drops of distillate was collected. Since the acetone test was still negative, it was concluded that reduction was complete. The residual isopropyl alcohol was removed *in vacuo*. The solid residue was cooled and then treated with 200 ml. of ice-cold 10% hydrochloric acid. This suspension was dissolved in about 375 ml. of water. With cooling and stirring, the aqueous solution was made strongly basic (pH about 12) with 40% potassium hydroxide. The basic solution was thoroughly extracted with ether, and the extract was washed with saturated sodium chloride solution and then dried with anhydrous sodium sulfate overnight. The ether extract was then filtered free of the drying agent and treated with anhydrous hydrogen chloride. An orange oil was thus produced. On standing for forty-eight hours in the refrigerator, this oil solidified. The solid was removed by filtration and washed with cold acetone; yield 10.4 Gm. (72%) of light yellow solid, m. p. 177–180°. After three recrystallizations from 95% ethyl alcohol-acetone, a sample melted at 180–181°.

The starting amino ketone hydrochloride melted at 187°, and the melting point of a mixture of the above product and starting material was 165–176°.

Anal. Calcd. for $C_{12}H_{18}N_2O_3 \cdot HCl$: C, 54.45, H, 6.68. Found: C, 54.42, H, 6.75.

Generally, previous workers employing this general procedure have used a Vigreux column (12), a modified Widmer column (13), or Hahn condenser (14). However, in the present studies, ordinary ground glass equipment providing for ready interchange of an upright condenser with one turned downward for distillation served admirably.

3-Dialkylamino-1-phenyl-1-propanols (Table III).—These compounds, synthesized by means of the foregoing procedure, are listed in Table III. Isolated as the hydrochlorides, they were recrystallized from alcohol-acetone mixtures. In each case, admixtures of the product with starting material caused a depression in melting point, indicating that the reduction was successful.

3-Dimethylamino-1-phenyl-1-propanol Hydrochloride (Table III, Compound 1).—This substance was synthesized through the foregoing method using aluminum isopropoxide and by Mannich and Heilner though catalytic hydrogenation, as indicated in Table I. The following sodium borohydride method has also been employed. It is based upon the general procedure of Chaikin and Brown (15).

A solution of 1.85 Gm. (0.05 mole) of sodium borohydride in 100 ml. of 50% methyl alcohol, kept at 20°, was placed in a 300-ml. round-bottomed flask, equipped with a stirrer, reflux condenser, and dropping funnel. Meanwhile, 10.6 Gm. (0.05 mole) of β-dimethylaminopropiophenone hydrochloride (Table III, footnote *a*) was dissolved in a minimum volume of water, and the solution made basic with cold 20% sodium hydroxide. Ether extraction of the free base, removal of the ether, and addition of 75 ml. of methyl alcohol was followed by dropwise addition of the prepared solution to the ice-cooled sodium borohydride solution so as to maintain the temperature below 30°. After the addition was complete, the mixture was warmed to 45–50° in order to decompose the excess sodium borohydride. The solvent was removed *in vacuo* and the residue made basic with 6 *N* sodium hydroxide and extracted with ether. After drying over anhydrous sodium sulfate, hydrogen chloride gas was passed into the filtered ether solution to precipitate the amine hydrochloride. Yield 7.2 Gm. (67%), m. p. 128–132°. After recrystallization from acetone, the compound melted at 133–134°. There was no depression of melting point upon admixture with samples from other preparative methods.

α-Bromo-β-dialkylaminopropiophenones (Table IV).—The compounds of Table IV were prepared by the bromination of the appropriate ketonic Mannich base hydrobromides in glacial acetic acid, using the procedure of Land, Ziegler, and Sprague (10). In one case (compound 3) the hydrochloride was used. Compounds 1 and 3 were recrystallized from isopropyl alcohol; alcohol was used for all others.

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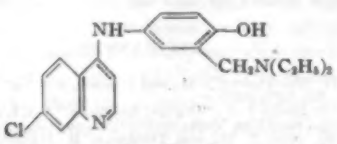
Antiamebic Agents IV.* Quinolines and Acridines with Amino-Hydroxyalkylanilino Side Chains†

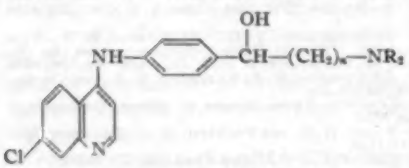
By W. LEWIS NOBLES,‡ VERLIN C. STEPHENS,§ LING WEI,‖ and J. H. BURCKHALTER

Six quinolines and five acridines containing side chains incorporating some of the structural features of amodiaquin and SN-8,137 were synthesized as potential antiamebic or antimalarial agents. The antiamebic activity of the compounds tested was insufficient to offer promise. Two possessed antimalarial activity in chicks greater than quinine, but insufficient for further interest.

THE ACRIDINE ANTIMALARIAL, quinacrine, and the quinoline antimalarials, chloroquine and amodiaquin (Camoquin®), have shown activity against extra-intestinal amebiasis in hamsters and thus have been used for extra-intestinal amebiasis (1). As a part of a search for compounds with improved antiamebic activity, it was decided to introduce certain chemical alterations into these antimalarial types.



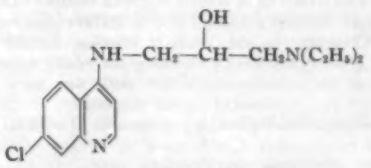
Amodiaquin



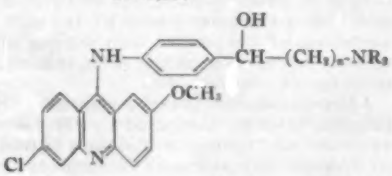
	n	NR_2
I	1	Amino
II	1	Piperidyl
III	2	Dimethylamino
IV	2	Diethylamino
V	2	Pyrrolidyl
VI	2	Piperidyl

Owing to the presence of the benzene ring as an integral part of the side chain of the antimalarial amodiaquin and the alcoholic hydroxyl as a favorable grouping in the antimalarial SN-8,137 (2), it appeared worth while to synthesize a number of agents (I–VI, inclusive) which would incorporate these special features of amodiaquin and SN-8,137.

Further, in view of the amebacidal effect of quinacrine, a few analogous acridines (VII, VIII, IX, and X) possessing the same side chains were also planned. Another one, indicated in the Experimental section as XI, differs from the other acridines in having a dimethylaminoalkanol chain attached *meta* to the benzene ring.

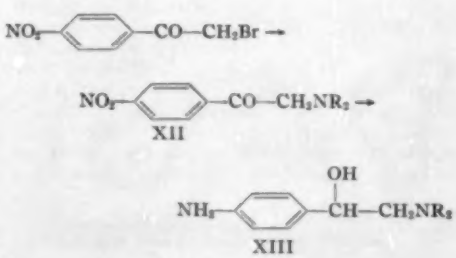


SN-8,137



	n	NR_2
VII	1	Amino
VIII	1	Piperidyl
IX	2	Diethylamino
X	2	Pyrrolidyl

The synthetic scheme used for compounds I, II, VII, and VIII follows:



* For paper III, see J. H. Burckhalter, *et al.*, *J. Am. Chem. Soc.*, **76**, 4902(1954).

† Received July 26, 1957, from the Laboratory of Pharmaceutical Chemistry, University of Kansas School of Pharmacy, Lawrence, Kan.

Portions of this paper were extracted from sections of the Ph. D. thesis of W. Lewis Nobles, 1952, and the M. S. thesis of Ling Wei, 1950.

‡ Fellow of The American Foundation for Pharmaceutical Education. Present address: School of Pharmacy, University of Mississippi, University, Miss.

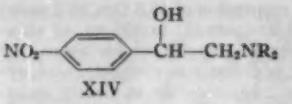
§ Parke, Davis and Co. Postdoctorate Fellow. Present address: Eli Lilly and Co., Indianapolis, Ind.

‖ Parke, Davis and Co. Research Assistantship. Present address: Johnson and Johnson, Research Center, New Brunswick, N. J.

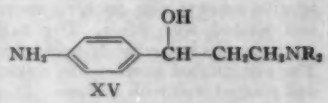
Starting material *p*-nitrophenacyl bromide, was available, since it is required in one of the chemical processes for chloramphenicol. The general procedure, adapted from the literature (3), consisted of mixing *p*-nitrophenacyl bromide with two equivalents of secondary amine in benzene or ether solution (4), allowing the mixture to stand for several hours, removing the precipitated by-product amine hydrobromide, drying the solution of the product over an inorganic drying agent, and finally precipitating the product (XII) as the hydrochloride. In practice, the by-product amine hydrobromide was obtained in nearly quantitative yield, but only a brown tarry product resulted. It was eventually found that the desired *p*-nitro-α-(1-piperidyl)-acetophenone could be prepared if the reaction were allowed to proceed for only a short while, and the hydrochloride were formed as quickly as possible to prevent degradation of the desired product. Also, a nitrogen atmosphere appeared to be favorable. Under such circumstances, it is not surprising that the analytical data of the piperidyl and diethyl intermediates (XII*a* and *b*) were not entirely satisfactory. In the case of XII*b*, decomposition during recrystallization occurred even though it was in the form of the hydrochloride; therefore, it was not employed for further synthesis.

p-Nitro-α-aminoacetophenone, the third intermediate of the type, has already been prepared by Long and Troutman (3). Its reduction and that of *p*-nitro-α-(1-piperidylamino)-acetophenone took place readily in very dilute hydrochloric acid to the diamines (XIII) by using platinum oxide catalyst and about three atmospheres of hydrogen. In the case of the piperidyl compound, it was necessary to keep the aqueous solution warm by means of a heat lamp to avoid the precipitation of an intermediate product, which prevented further reduction. Isolation of the intermediate diamine hydrochlorides was not feasible because of apparent decomposition in the air, but isolation was actually unnecessary since, after removal of the catalyst by filtration, the solution possessed the proper pH for reactivity with either 4,7-dichloroquinoline or 6,9-dichloro-2-methoxyacridine. This procedure has been successfully employed before for the synthesis of amodiaquin type compounds (5).

For the synthesis of the quinolines (III–VI inclusive) and acridines (IX and XI), 3-dialkylamino-1-(nitrophenyl)-1-propanols (XIV), made available from previous studies (6), were reduced catalytically to the diamino alcohols (XV), which were allowed to react directly, as in the case of



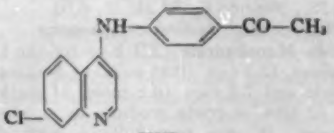
XIV



XV

XIII, with either 4,7-dichloroquinoline or 6,9-dichloro-2-methoxyacridine.

Earlier, unsuccessful attempts to obtain quinolines of the type III to VI were made through preparation of 4-(7-chloro-4-quinolylamino)-acetophenone (XVI), as well as the isomeric 3-substituted acetophenone (XVII). Excellent yields



XVI

of XVI or XVII were obtained from a condensation of 4,7-dichloroquinoline and *p*- or *m*-aminoacetophenone in an acidic medium. However, these substituted ketones could not be made to undergo the Mannich reaction to give the amino ketones required for the synthesis of Type III-VI compounds.

Pharmacological Results.[1]—Quinolines I, II, and VI were active *in vitro* against *E. histolytica*, but only at 1:500 dilution. Tested in rats as a possible intestinal amebacide, I was found to be slightly active but not promising. Against hepatic amebiasis in hamsters, I was less active than chloroquine. Acridines VII, VIII, IX, X, and XI showed *in vitro* activity at 1:500 dilution, and *in vivo* in rats, the same compounds were described "as active but not promising."

Quinoline I and acridines VII, IX, X, and XI had quinine equivalents (quinine = 1) of, respectively, 2.4, 0.45, 1.5, 0.8, and 0.3 against *Plasmodium lophurae* in chicks, but these activities were not considered to be of interest when compared with currently accepted antimalarial drugs.

EXPERIMENTAL[2]

p-Nitro-α-(1-piperidyl)-acetophenone Hydrochloride Monohydrate (XIIa).—A solution of 34 Gm. (0.4 mole) of piperidine in 75 ml. of absolute ether was added dropwise, with vigorous stirring and under a slow stream of nitrogen, over a period

[1] The authors are grateful to Dr. Paul E. Thompson of the Parke, Davis & Co. Research Division for the pharmacological results.

[2] Melting points are uncorrected.

of a half hour to a suspension of 48.8 Gm. (0.2 mole) of *p*-nitrophenacyl bromide (3) in 400 ml. of absolute ether. Stirring was continued for about five minutes after the addition was complete, and by-product piperidine hydrochloride in nearly quantitative amount was quickly removed by filtration and washed with ether. Dry hydrogen chloride in excess was immediately bubbled into the filtrate. The precipitated product was collected on a funnel and washed well with acetone. It was air dried to give 51 Gm. (84% yield) of crude material which melted at 160–166° decompn. By recrystallization from 200 ml. of methanol, 39 Gm. (65% yield) of product was obtained, m. p. 177–181° decompn. Further recrystallization from methanol-acetone did not alter the melting point.

An attempt to analyze this compound for ionic chloride using silver nitrate resulted in the formation of free silver.

Anal. Calcd. for $C_{13}H_{18}N_2O_3.HCl.H_2O$: C, 51.57; H, 6.32. Found: C, 52.25; H, 6.60.

p-Nitro-α-diethylaminoacetophenone Hydrochloride Monohydrate (XII b).—By the foregoing procedure, 12.2 Gm. (0.05 mole) of *p*-nitrophenacyl bromide and 7.3 Gm. (0.1 mole) of diethylamine gave 12 Gm. of crude product, m. p. about 150° decompn. It was recrystallized with difficulty from a methanol-acetone mixture and from isopropyl alcohol. After four recrystallizations, it melted at 165–167° decompn. Decomposition appeared to occur readily in hot solvents.

Anal.: Calcd. for $C_{12}H_{16}N_2O_3.HCl.H_2O$: C, 49.57; H, 6.59. Found: C, 50.01; H, 6.00.

Reduction to Diamines (Types XIII and XV).—A suspension of 12 Gm. (0.056 mole) of *p*-nitro-α-aminoacetophenone hydrochloride (3) in a solution of 6 ml. of hydrochloric acid and 200 ml. of water was warmed and then subjected to platinum oxide reduction in a Parr apparatus. Within two hours the theoretical amount of hydrogen was usually absorbed. The catalyst was then removed by filtration, and the filtrate used directly in subsequent reactions.

Reduction of the piperidyl analog (XIIa) was carried out in the same fashion except that it was necessary to keep the suspension hot during the reduction by means of a heat lamp, in order to prevent precipitation of an intermediate product.

Type XIV intermediates in the form of the hydrochlorides (6) were suspended in alcohol and reduced catalytically using Adams' catalyst to type XV diamines.

Compounds from Type XII Intermediates

2-Amino-1-[*p*-(7-Chloro-4-quinolylamino)-phenyl]-1-ethanol Dihydrochloride (I).—The filtrate from the reduction of *p*-nitro-α-aminoacetophenone, containing diamine (XIII) in acid solution, and 10 Gm. (0.05 mole) of 4,7-dichloroquinoline was heated on steam bath for thirty minutes, according to the general procedure of Burckhalter, *et al.* (5). After filtration, the solution was concentrated to a thick brown sirup. It was then warmed briefly with absolute alcohol and the solution allowed to cool overnight to give a yellow product, 14.5 Gm. (73% yield), m. p. >300°. It was recrystallized from methanol.

Anal.: Calcd. for $C_{17}H_{16}Cl.N_3O.2HCl.{}^1/_3H_2O$: C, 51.60; H, 4.84. Found: C, 52.02; H, 5.06.

2-Amino-1-[*p*-(6-chloro-2-methoxy-9-acryidylamino)-phenyl]-1-ethanol Dihydrochloride (VII).—The filtrate from the reduction of 12 Gm. of *p*-nitro-α-aminoacetophenone hydrochloride was heated with an alcohol suspension of 6,9-dichloro-2-methoxyacridine, following the foregoing general procedure. However, concentration of the filtrate was not necessary in giving 21 Gm. (80% yield) of bright orange-colored product, m. p. >300°. Recrystallization from very dilute hydrochloric acid[1] yielded 18 Gm. (70%) of product.

Anal.: Calcd. for $C_{22}H_{20}ClN_3O_2.2HCl.2H_2O$: C, 50.73; H, 5.42. Found: C, 50.35; H, 5.66.

2-1′-Piperidyl-1-[*p*-(7-chloro-4-quinolylamino)-phenyl]-1-ethanol (II).—The filtrate from the reduction of 15.1 Gm. (0.05 mole) of XII *b* was treated with 0.05 mole of 4,7-dichloroquinoline, as in the production of I. When no product precipitated from the reaction mixture, it was rendered basic with ammonia solution. The resulting precipitate was collected, washed with water and then acetone to remove any unreacted dichloroquinoline. Two recrystallizations of an impure product gave 8 Gm. (42% yield) of white base (II), m. p. 191–192°.

Anal.: Calcd. for $C_{23}H_{24}ClN_3O$: C, 69.19; H, 6.33. Found: C, 68.72; H, 6.74.

2-1′-Piperidyl-1-[*p*-(6-chloro-2-methoxy-9-acridylamino)-phenyl]-1-ethanol Dihydrochloride(VIII).—The filtrate from the reduction of 14.2 Gm. (0.047 mole) of XII*b* was treated with 0.05 mole of the chloro acridine, as in VII, to give 22.5 Gm. (90% yield) of product, m. p. 195–200° decompn. Recrystallization from 2% hydrochloric acid[1] gave 18 Gm. (72% yield) of bright red II, m. p. 207–210°. After two further recrystallizations, m. p. was 210–213°. For analysis, a small sample was dried for three days over phosphorus pentoxide.

Anal.: Calcd. for $C_{27}H_{28}ClN_3O_2.2HCl$: Cl, 13.26, Found: Cl, 13.24.

Compounds from Type XIV Intermediates

3-Dimethylamino-1-[*p*-(7-chloro-4-quinolylamino)-phenyl]-1-propanol (III).—Three grams (0.011 mole) of 3-dimethylamino-1-(*p*-nitrophenyl)-propanol hydrochloride (6) was suspended in absolute alcohol and reduced catalytically using Adams' catalyst. The clear solution thus obtained was treated with a slight excess of alcoholic hydrogen chloride and then filtered to remove the catalyst. An equivalent amount, 2.2 Gm. (0.011 mole), of 4,7-dichloroquinoline was added to the filtrate and the resulting mixture was heated at reflux temperature for about two hours, during which time a clear solution resulted. The reaction mixture was made basic with 28% ammonium hydroxide and 4 Gm. (78% yield) of a light yellow solid precipitated, m. p. 207–212° decompn. It was recrystallized from dilute alcohol, m. p. 218–220° decompn.

Anal. Calcd. for $C_{20}H_{22}ClN_3O$: C, 67.49; H, 6.23. Found: C, 68.07; H, 6.77.

3-Diethylamino-1-[*p*-(7-chloro-4-quinolylamino)-phenyl]-1-propanol (IV).—Using the foregoing procedure, the appropriate reactants (6) gave 58% of light yellow solid (IV), m. p. 199–201° decompn. (203–204 decompn. after recrystallization from dilute alcohol).

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[1] Recrystallization from water is possible but the presence of acid facilitates filtration and drying.

Anal.: Calcd. for $C_{22}H_{28}ClN_3O$: C, 68.82; H, 6.83. Found: C, 68.20; H, 7.19.

3-Diethylamino-1-[*p*-(6-chloro-2-methoxy-9-acridylamino)-phenyl]-1-propanol Dihydrochloride Monohydrate (IX).—Using the procedure of III, except that the hydrochloride was isolated upon cooling the solution after reduction, the appropriate reactants (6) gave 69% yield of orange-colored IX, m. p. 192–195° decompn. (195–197° decompn. after recrystallization from alcohol-acetone).

Anal. Calcd. for $C_{27}H_{29}ClN_3O_2.2HCl.H_2O$: C, 58.43; H, 6.18. Found: C, 58.30; H, 6.48.

3-1′-Pyrrolidyl-1-[*p*-(7-chloro-4-quinolylamino)-phenyl]-1-propanol Dihydrochloride Hydrate (V).—Using the procedure of III, the appropriate reactants (6) gave 82% yield of crude free base of V, m. p. 188–191° decompn. When recrystallization from dilute alcohol failed to yield an analytically pure sample, the compound was dissolved in dry ether. An ether-insoluble portion was removed by filtration, and the hydrochloride was precipitated by passage of dry hydrogen chloride into the solution. By dissolving in alcohol and reprecipitating through the addition of acetone, the flocculent precipitate was crystallized. Two recrystallizations from alcohol-acetone gave white product V, m. p. 141–146° decompn.

Anal.: Calcd. for $C_{25}H_{24}ClN_3O.2HCl.2^1/_2H_2O$: C, 52.85; H, 6.26. Found: C, 52.52; H, 6.68.

3-1′-Pyrrolidyl-1-[*p*-(6-chloro-2-methoxy-9-acridylamino)-phenyl]-1-propanol Dihydrochloride (X).—Using the procedure of III except that the hydrochloride was isolated upon cooling the solution after reduction, the appropriate reactants (6) gave 76% yield of orange-colored X, m. p. 190–193° decompn. Recrystallized from alcohol-acetone, it melted at 194–195° decompn.

Anal.: Calcd. for $C_{27}H_{29}ClN_4O_2.2HCl.1^1/_2H_2O$: C, 57.70; H, 6.01. Found: C, 57.60; H, 6.38.

3-1′-Piperidyl-1-[*p*-(7-chloro-4-quinolylamino)-phenyl]-1-propanol Dihydrochloride (VI).—Using the procedure of III and the appropriate intermediates (6), the product was obtained in the form of an amorphous mass which could not readily be crystallized. The material was dissolved in ether, the ether solution dried over potassium carbonate, and the hydrochloride made by passing dry hydrogen chloride into the filtered ether solution. The yellow crystallizing precipitate VI was obtained in 46% yield, m. p. 278–281° decompn. Recrystallized from alcohol-acetone, it melted at 283–284° decompn.

Anal.: Calcd. for $C_{23}H_{26}ClN_3O.2HCl$: C, 58.92; H, 6.02. Found: C, 58.67; H, 6.12.

3-Dimethylamino-1-[*m*-(6-chloro-2-methoxy-9-acridylamino)-phenyl]-1-propanol Dihydrochloride (XI).—Using the procedure of III except that the hydrochloride was isolated upon cooling the solution after reduction, the appropriate reactants (6) gave 67% yield of orange-colored XI, m. p. 174–178° decompn. It was recrystallized from alcohol-acetone, m. p. 179–180° decompn.

Anal.: Calcd. for $C_{25}H_{25}ClN_4O_2.2HCl.2^1/_2H_2O$: C, 54.30; H, 6.07. Found: C, 54.20; H, 6.00.

4-(7-Chloro-4-quinolylamino(-acetophenone (XVI).—A mixture of 13.5 Gm. (0.1 mole) of *p*-aminoacetophenone, 19.8 Gm. (0.1 mole) of 4,7-dichloroquinoline, 100 ml. of alcohol, and 0.5 ml. of concentrated hydrochloric acid was heated at reflux temperature for two hours. After cooling the reaction mixture, the bright yellow product (XVI) hydrochloride was isolated in nearly quantitative yield, m. p. 272–273° decompn. Recrystallization from alcohol changed the melting point to 281–282° decompn.

Anal.: Calcd. for $C_{17}H_{13}ClN_2O.HCl$: C, 61.23; H, 4.20. Found: C, 61.57; H, 4.12.

A quantity of the hydrochloride was treated while stirring with excess alcoholic ammonium hydroxide solution to give the free base XVI. The product was collected and washed thoroughly with distilled water until chloride ion was absent from the filtrate. Recrystallized from methyl alcohol, the yellowish XVI was obtained in 88% yield, m. p. 222°.

Anal.: Calcd. for $C_{17}H_{13}ClNO.^1/_2H_2O$: C, 66.81; H, 4.62. Found: C, 66.50; H, 4.42.

3-(7-Chloro-4-quinolylamino)-acetophenone (XVII).—Employing *m*-aminoacetophenone in place of its para-isomer in the foregoing procedure, a 90% yield of yellow (XVII) hydrochloride was obtained, m. p. 301° decompn.

Anal.: Calcd. for $C_{17}H_{13}ClN_2O.HCl$: Cl(ionic), 10.65. Found: Cl, 10.52.

The hydrochloride was converted to the yellowish free base XVII, as with XVI in 93% yield, m.p. 190°.

Anal.: Calcd. for $C_{17}H_{13}ClN_2O$: C, 68.79; H, 4.42. Found: C, 68.41; H, 4.40.

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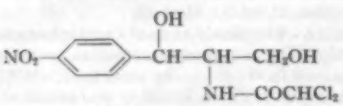
Attempted Synthesis of a Vinylog of Chloramphenicol*

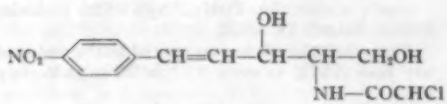
By W. LEWIS NOBLES† and J. H. BURCKHALTER

Applying essentially the synthetic procedures of Long and Troutman, which led to chloramphenicol, we have attempted to synthesize a vinylog of the antibiotic in order to study the application of the theory of vinylogy to pharmacological results. However, failure to achieve the desired synthesis has suggested that even chemically the theory does not hold entirely, especially in regard to an apparently high specificity of structure which allows monohydroxymethylation in certain chloramphenicol intermediates and not in their vinylogs.

IT IS WELL KNOWN that the chemical reactivity of a compound and its vinylog may be similar (1). In further study of the theory that a vinylog may possess pharmacological action similar to the parent drug (2), we proposed to attempt the synthesis of a vinylog (I) of chloramphenicol, a structure differing from the antibiotic only by a vinyl grouping. Although the synthesis of I was not achieved, we wish to report the results which were obtained.

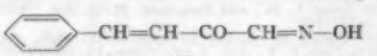


Chloramphenicol

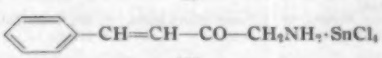


I

The synthetic scheme was patterned after the approach of Long and Troutman to chloroamphenicol (3). The early styryl intermediates had been made over fifty years ago by Sir Robert Robinson for an entirely different purpose (4). The latter prepared oximinomethyl styryl ketone (II) from benzalacetone and isoamylnitrile, and then reduced it with stannous chloride and hydrochloric acid to the stannichloride complex (III). However, it was found that the previously developed procedures, while satisfactory for small quantities of reactants, were unsuited for larger amounts. For example, in the preparation of II, a temperature as low as 20° was necessary, yet near that temperature benzalacetone crystallized from solution. But, if the solution of benzalacetone in Skelly B was prepared by melting the ketone and adding it to the solvent on the night prior to the morning during which the reaction was carried out, the solution would cool slowly to room temperature (16–20°) without depositing crystals of benzalacetone. Further, in contrast with the original work involving smaller quantities (4), in order to prevent decomposition of II, it was important to keep the mixture cooled with stirring for two hours after all the reagent had been added, instead of allowing it to stand at room temperature.



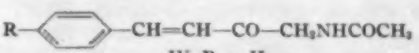
II



III

The stannous chloride reduction of oxime II to III, employing the original procedure (4), afforded difficulty. In our hands, direct addition of II to stannous chloride in acid caused the evolution of considerable heat and resulted in the formation of large, dark lumps of solid which were difficult to purify. Upon the suggestion of Dr. S. H. Johnson, of This Laboratory, these difficulties were obviated by adding the oxime as an alcoholic solution.

Acetylation of the tin complex salt (III) in acetic acid using sodium acetate as a buffer, as prescribed for its benzoylation (4), and employing acetic anhydride or acetyl chloride or mixtures of the two, gave only small amounts of IV. However, the procedure of Long and Troutman (3) using sodium hydroxide and acetic anhydride resulted in consistently good yields of 1-acetamido-4-phenyl-3-buten-2-one (IV). This compound was then nitrated to give a 50% yield of V.



IV, R = H
V, R = NO_2

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Extracted from a portion of the Ph. D. thesis of W. Lewis Nobles, 1952.

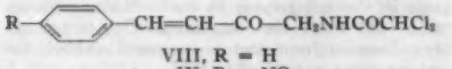
† Fellow of the American Foundation for Pharmaceutical Education. Present address, School of Pharmacy, University of Mississippi, University, Miss.

Unlike the nitration of benzalacetone (2), only the *para*-isomer was isolated.

Attempts to hydroxymethylate V resulted only in the recovery of starting material. This might be attributed to the lack of solubility of V in solvents customarily used in the reaction. While V is soluble in boiling alcohol, Long and Troutman (3) have indicated that the temperature should be kept between 35 and 40° for similar hydroxymethylations.

In view of the failure to hydroxymethylate V, attempts were made to introduce the grouping into IV which lacks the nitro group and which is more soluble in the required solvents. It was thought that the diacetyl derivative could then be para-nitrated in a step toward obtaining I. However, no less than thirty unsuccessful attempts were made to monohydroxymethylate IV. The use of sodium or potassium carbonate as catalyst at room temperature led to no reaction. When the temperature was raised to 50–60°, the bis-methane structure was apparently formed. The product of the reaction using sodium bicarbonate at 35–40° was apparently the substance containing two hydroxymethyl groups. The use of one, two, or four equivalents of formaldehyde still gave the same compound. Other workers,notably Pain and Slack (5) and Hays and associates (6), have commented upon the difficulty of attaining such monomethylol derivatives. The latter workers concluded that the reaction will not as a general rule yield them. Our results are in accord with this conclusion.

Still another approach, patterned after the studies of Long and Troutman (7), was followed. IV and V were hydrolyzed in dilute hydrochloric acid to the corresponding amine hydrochlorides. The yields were neither good nor consistent, and considerable decomposition occurred. Nevertheless, these salts, designated as VI and VII in the Experimental section, were treated with dichloroacetyl chloride in the presence of triethylamine to yield VIII and IX. Though a satis-



VIII, R = H
IX, R = NO_2

factory elementary analysis of IX was not obtained, infrared studies of the compound conducted by Mr. Bruce Scott and Dr. George Moersch, of Parke, Davis & Co., Research Division, indicated the structure to be correct. The analytical data suggest that the starting material dichloroacetyl chloride was contaminated with monochloroacetyl chloride.

Finally, an attempt was made to monohydroxymethylate VIII. Here, unlike the previous experiments, the desired derivative (X) was obtained. But, in view of the poor yields obtained in the sequence of reactions, it was decided to discontinue the studies at this point.

Since the desired vinylog of chloramphenicol was not obtained, conclusions based upon pharmacological activity are impossible to draw. However, in regard to chemical adherence to the theory of vinylogy, it is felt that there was a lack of complete conformity because of our failure to monohydroxymethylate vinylogs IV and V, whereas the same conditions have been shown to give positive results with the parent compounds (3).

EXPERIMENTAL[1]

Oximinomethyl Styryl Ketone (II).—Into a three-liter, three-necked flask equipped with a mechanical stirrer was placed 1.5 liters of Skelly Solve B. One hundred grams (0.69 mole) of benzalacetone was melted on the steam bath and dissolved therein. The flask was allowed to come slowly to room temperature, surrounded by a water bath. To this solution was then added 225 ml. of isoamyl nitrite (freshly prepared) and 40 ml. of concentrated hydrochloric acid in alternate portions. The temperature was maintained near 20° by the addition of small amounts of ice to the surrounding water bath. After the addition was complete, the flask was surrounded by an ice-salt bath and stirring continued for another two hours. The solid thus obtained was removed by filtration and washed alternately with benzene and water until the washings were colorless. The weight of air-dried material was 82 Gm. (65% yield). The product was recrystallized from methyl alcohol-water to yield a solid that melted at 143°. Robinson and Foulds (4) reported no melting point for this compound. In regard to yield, they indicated only that it was "excellent." Recrystallization was not routinely employed, since experience indicated that the air-dried material was pure enough for the subsequent reaction.

Aminomethyl Styryl Ketone Stannichloride (III).—Oximino styryl methyl ketone (40 Gm., 0.23 mole) was dissolved in 95% ethyl alcohol. A solution of 110 Gm. of stannous chloride in 300 ml. of concentrated hydrochloric acid was prepared, cooled and placed in a one-liter, three-necked flask equipped with a mechanical stirrer. The flask was surrounded by an ice bath and the alcoholic solution of the oxime added slowly with stirring. Upon the completion of the addition, the ice bath was removed and the stirring continued for six hours. The solid was removed by filtration and washed thoroughly with alcoholic hydrogen chloride. A light tan solid was obtained upon air drying this material. Eighty-two grams (80% yield) of the tin complex was thus obtained.

1-Acetamido-4-phenyl-3-buten-2-one (IV).—To a three-liter flask equipped with an efficient stirrer, a thermometer, and a dropping funnel, was added the

[1] Melting points are uncorrected.

tin complex salt obtained from 35 Gm. (0.2 mole) of oximinomethyl styryl ketone and 250 ml. of hot water. The mixture was stirred until a clear solution was produced. The flask was then cooled to −5°. Twenty-five grams of acetic anhydride was added in one portion, and the resulting mixture stirred rapidly while a cold solution of 42 Gm. of sodium hydroxide in 100 ml. of water was added at such a rate as to keep the temperature below 10°. When about 75 ml. of the sodium hydroxide solution had been added and the mixture was almost alkaline, 7 Gm. of acetic anhydride was poured in. Addition of the hydroxide was completed and stirring maintained for thirty minutes at 0°.

The mixture was saturated with sodium chloride and extracted five times with 500-ml. portions of ethyl acetate. The combined extracts were washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated to 200 ml. by removal of solvent. To the hot concentrate was added 180 ml. of Skelly B. The mixture was cooled to 5° and filtered. The yield was 34 Gm. (81%), m. p. 118–123°. An analytical sample was obtained by recrystallization from water. It melted at 127°.

Anal. Calcd. for $C_{12}H_{12}NO_2$: C, 71.90; H, 6.45. Found: C, 71.74; H, 6.36.

1-Acetamido-4-(*p*-nitrophenyl)-3-buten-2-one (V).—To a 200-ml., three-necked flask, equipped with a mechanical stirrer and thermometer, was added 60 ml. of sulfuric acid. The flask was surrounded by an acetone-dry ice bath and the temperature lowered to −20° by the addition of pieces of dry ice. To this was then slowly added 20.3 Gm. (0.1 mole) of IV, care being taken to insure that the temperature did not rise above −10° during the addition. When all the solid had been added and the temperature again lowered to −20°, a nitrating solution of 6.4 ml. of concentrated nitric acid (sp. gr. 1.42) and 10 ml. of concentrated sulfuric acid was added dropwise. The mixture in the flask became rather dark and very viscous. The mixture was stirred for fifteen minutes after the addition of the last portion of acid, the cooling bath having been removed during this period of stirring. The mixture was then poured into one liter of cracked ice and water, and stirred for two hours. A yellow, rather pasty solid formed at first, but this soon became more granular in appearance. The solid was removed by filtration and washed with water to remove the traces of acid present. It was then allowed to air dry overnight; yield 12.2 Gm. (50%), m. p. 146–155°. A sample for analysis was purified by repeated recrystallization from ethyl acetate-Skelly B. The purified product is fluffy in appearance and light yellow in color, m. p. 171°.

Anal. Calcd. for $C_{12}H_{12}N_2O_4$: C, 58.05; H, 4.87. Found: C, 58.32; H, 5.01.

Reactions of 1-Acetamido-4-phenyl-3-buten-2-one (IV) with Formaldehyde.—The general procedure in all cases was to dissolve or suspend the amide in methyl alcohol, ethyl alcohol or a mixture of the two, with or without the addition of a few drops of water, and then to add the appropriate quantity of aqueous formaldehyde (37%) or paraformaldehyde. A trace of the desired catalyst was then added and the suspension heated and stirred for varying periods. Then the reaction mixtures were poured into ice water and the products collected and examined. The amide was recovered unchanged after many experiments, but the conditions tabulated led to either the formation of a compound A, postulated as having the two active methylene hydrogens replaced by —CH_2OH groups or a compound B, believed to be of the bis-methane structure. (See tabulation below.)

For Compound A: C_6H_5—CH=CH—CO—C-$(NHCOCH_3)(CH_2OH)_2$:

Anal. Calcd. for $C_{14}H_{17}NO_4$: C, 63.86; H, 6.51. Found: C, 63.87; H, 6.61.

For Compound B: $[C_6H_5$—CH=CH—CO—CH-$(NHCOCH_3)]_2$—CH_2:

Anal. Calcd. for $C_{25}H_{28}N_2O_4 \cdot {}^1/_2H_2O$: C, 70.23; H, 6.40. Found: C, 70.25; H, 6.66.

1-Amino-4-phenyl-3-buten-2-one Hydrochloride (VI).—Eight and one-tenth grams (0.04 mole) of the corresponding acetamido derivative was heated for two hours on the steam bath with 100 ml. of 5% hydrochloric acid. The solid dissolved shortly after the heating began. The solution gradually became dark red in color and a dark oil formed on the bottom of the flask. The mixture was cooled slowly to room temperature and then the hydrolysate was twice extracted with ether to remove any neutral material. The aqueous solution of the hydrochloride was then taken to dryness *in vacuo*. The solid thus obtained was filtered and washed with acetone-ether; yield 3 Gm. (39%). The crude solid was dissolved in an acetone-isopropyl alcohol solution, treated with charcoal, filtered, and set aside in the refrigerator to cool. White crystals, m. p. 184–189°, were obtained. On further recrystallization from acetone-isopropyl alcohol, the melting point was raised to 193°.

Anal. Calcd. for $C_{10}H_{11}NO \cdot HCl$: Cl, 17.94. Found: Cl, 18.12.

1-Amino-4-(*p*-nitrophenyl-3-buten-2-one Hydrochloride (VII).—Seven and five-tenths grams (0.03 mole) of 1-acetamido-4-(*p*-nitrophenyl)-3-buten-2-one (V) was heated with 125 ml. of 5% hydrochloric acid on the steam bath for two and one-half hours. Slowly a red solution formed, but simultaneously a considerable amount of brown tar was deposited in the bottom of the flask. The mixture was filtered while warm and then set aside to cool. A yellow solid, 3 Gm. (41% yield), m. p. 262–266° (decompn.),

Catalyst	Solvent	Time, hr.	Equivalents of Formaldehyde	Product and Yield
$NaHCO_3$	CH_3OH—HOH	2	1	A (40%)
$NaHCO_3$	HOH	2	2	A (75–85%)
$NaHCO_3$	HOH	2	4	A (75–85%)
Na_2CO_3	CH_3OH	2	2	B (poor)
$NaHCO_3$	CH_3OH	12	2 (para-formal.)	B (poor)
K_2CO_3	C_2H_5OH	4	2	Undetermined

was thus obtained. This solid was then recrystallized from dilute ethyl alcohol to which a few drops of concentrated hydrochloric acid had been added. It melted at 276° (decompn.). The compound begins to darken and shrink in the region of 210–215°, but true melting does not occur until the higher temperature is reached.

Anal. Calcd. for $C_{10}H_{10}N_2O_2$·HCl: Cl, 14.61. Found: Cl, 14.74.

1-Dichloroacetamido-4-phenyl-3-buten-2-one (VIII).—Five and nine-tenths grams (0.03 mole) of VI and 5.1 Gm. (0.035 mole) of dichloroacetyl chloride were mixed, together with 100 ml. of dry benzene, in a 300-ml. flask equipped with a reflux condenser, mechanical stirrer, and dropping funnel. The mixture was cooled and stirred while 6.67 Gm. (0.67 mole) of triethylamine was added over a period of fifteen minutes. Toward the end of the addition of the amine, the mixture thickened and the cooling bath was removed. Stirring was continued for another hour. The mixture was then heated to reflux and filtered while hot. As the filtrate cooled a quantity of solid product separated and this was removed by filtration. The filtrate was then evaporated to dryness by passing a current of air over it. The residue thus obtained was washed first with Skelly B and then with water. This residue was then combined with the solid previously obtained by filtration and both were recrystallized from alcohol-water; yield 3.8 Gm. (47%), m. p. 128–131°. An analytical sample was obtained by two additional recrystallizations from benzene; m. p. 133–134°.

Anal. Calcd. for $C_{12}H_{11}Cl_2NO_2$: C, 52.96; H, 4.07. Found: C, 53.23; H, 4.13.

1-Dichloroacetamido-4-(*p*-nitrophenyl)-3-buten-2-one (IX).—Seven and one-half grams (0.03 mole) of VII and 5.1 Gm. (0.035 mole) of dichloroacetyl chloride were mixed together with 160 ml. of dry benzene in a 300-ml. flask equipped with a mechanical stirrer, dropping funnel, and reflux condenser. The mixture was surrounded by an ice bath and cooled and stirred while 6.7 Gm. (0.065 mole) of triethylamine was added over a period of twenty minutes. Stirring was continued for a total of two hours; the cooling bath having been removed toward the end of the addition of the triethylamine. The mixture was then heated to reflux and filtered hot. The filtrate was concentrated *in vacuo*. The residue was washed, first with Skelly B and then with water. It was dissolved in 95% ethyl alcohol by warming, treated with charcoal, and filtered while hot. The filtrate was made slightly turbid by the addition of a few drops of water. This was again filtered and set aside to cool. The yellow solid which formed was removed by filtration; yield 3 Gm. (30%), m. p. 178–182°. An analytical sample was prepared by repeated recrystallization from benzene.

Three different samples were prepared by the above method and each was analyzed. A typical result is given.

Anal. Calcd. for $C_{12}H_{10}Cl_2N_2O_4$: C, 45.44; H, 3.17; Cl, 22.36. Found: C, 46.35; H, 3.65; Cl, 20.15.

TABLE I.—INFRARED SPECTRAL DATA ON COMPOUND IX

Wavelength, μ, Absorption Peaks (IX)	Wavelength ranges, μ, of Compounds Contg. Similar Functional Groups[b]
N—H.....3.01	2.88–3.00
C=O, keto.....5.91	5.81–5.99
C=O, amide I[a]...6.00	5.99–6.25
C=C.....6.18	6.02–6.11
C_6H_5C=C.....6.28	6.30–6.35
Amide II[a].....6.45	6.39–6.67
Nitro I[a].....6.64	6.23–6.56
Nitro II[a].....7.45	7.20–7.60

[a] One of two characteristic bands in this region.
[b] As listed by H. M. Randall, *et al.*, in "Infrared Determination of Organic Substances," D. Van Nostrand Co., Inc., New York, 1949, p. 20.

In addition to this information, Dr. Moersch of Parke, Davis and Co. stated that the ultraviolet examination of IX gives an absorption very similar to *p*-nitrocinnamaldehyde, which would be expected for a structure of this type.

2-Dichloroacetamido-1-hydroxy-5-phenyl-4-penten-3-one (X).—A mixture of 1.35 Gm. (0.005 mole) of VIII, 10 ml. of 95% ethyl alcohol, and 1.75 ml. (*ca.* 0.025 mole) of 37% aqueous formaldehyde was placed in a 25 ml. round-bottomed flask equipped with a thermometer. The mixture was agitated by means of a magnetic stirrer. One-tenth gram of sodium bicarbonate was added and the mixture stirred and warmed to 35°. Soon a clear solution was formed. Stirring was continued for one and one-half hours, at the end of which time the solution was still clear. The solution was then poured into 25 ml. of ice and water. A gummy solid soon began to separate. The flask was then refrigerated for forty-eight hours, during which time the gummy mass solidified. The solid was removed by filtration, yield 0.9 Gm. (64%). This was recrystallized from benzene to give a solid, m. p. 118–120°. On further recrystallization from benzene, the melting point was raised to 122°.

Anal. Calcd. for $C_{12}H_{11}Cl_2NO_3$: C, 51.67; H, 4.34. Found: C, 51.96; H, 4.41.

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Further Studies on *l*-Arterenol Tachyphylaxis in the Isolated *Venus mercenaria* Heart*

By TSUNEO FUJITA† and DAVID E. MANN, JR.‡

A TACHYPHYLACTIC RESPONSE to a pharmacological agent is characterized by a diminished response to successive doses of the same agent within a limited time interval. This phenomenon is inherent to a relatively small number of drugs, among which are renin (1), posterior pituitary extracts (2), atropine (3), and various sympathomimetic amines (4). According to Brown (5), there is general agreement that tachyphylaxis does not occur to the pressor response evoked by *l*-arterenol. However, Pless and Mann (6) observed a tachyphylactic response in the isolated *Venus mercenaria* heart upon successive administrations of *l*-arterenol.

These investigators noted that the inhibitory response to *l*-arterenol was decreasingly inhibited upon the first two administrations, with no response to the third. When a fresh heart was immersed in sea water in which tachyphylaxis had been previously demonstrated, the heart failed to respond to the initial dose of *l*-arterenol. The tachyphylactic response was attributed to the presence of oxidation product(s) of *l*-arterenol since a pink discoloration of the sea water was noted coincident with the onset of tachyphylaxis. They also noted that pretreatment with varying concentrations of ephedrine sulfate altered the response to *l*-arterenol, and with sufficiently high concentrations, the response to *l*-arterenol was completely blocked.

Further investigation of the responses of the *Venus* heart to *l*-arterenol was undertaken to confirm these observations.

EXPERIMENTAL

The soft parts of the clam were exposed dorsally by first cracking the shell with a gentle hammer blow, severing the adductor muscles, and removing the umbo and hinge of the valves. By careful dissection of the mantle and pericardium, the single median ventricle and one of the two laterally-disposed, thin-walled auricles were exposed. The auricle was severed after a threaded, size-zero fishhook was passed through the ventricle at the

TABLE I.—PER CENT INHIBITION OF THE PREINJECTION AMPLITUDE OF THE *Venus* HEART AFTER THE ADMINISTRATION OF *l*-ARTERENOL CLASSIFIED ACCORDING TO TYPE OF RESPONSE

No. Detn.	Type I — Concn. *l*-Arterenol 1×10^{-3}	2×10^{-3}	3×10^{-3}	4×10^{-3}
1	25	24	0	..
2	29	18	0	..
3	26	17	0	..
4	34	24	0	..
5	34	70	0	..
6	36	91	0	..
7	47	31	0	..
8	49	29	0	..
9	49	45	0	..
10	61	54	0	..
11	65	36	0	..
12	20	12	8	0
13	22	44	12	0
14	22	44	14	0
15	38	21	2	0
16	50	40	38	0
17	54	39	23	0
18	78	24	30	0
19	29	9	14	..
20	22	24	8	..
21	19	8	8	..
22	67	17	10	..
23	50	44	23	26
24	38	36	34	..

No. Detn.	Type II — Concn. *l*-Arterenol 1×10^{-3}	2×10^{-3}	3×10^{-3}	4×10^{-3}
1	20	10	0	..
2	20	16	0	..
3	26	47	0	..
4	33	32	0	..
5	35	30	0	..
6	47	50	0	..
7	50	37	0	..
8	58	15	0	..
9	60	29	0	..
10	72	40	0	..
11	80	58	0	..
12	53	24	26	0
13	62	87	24	..
14	31	21	11	6
	Type III			
1	17	15	0	..
2	50	23	0	..
3	24	34	40	0
4	32	22	21	0
5	20	24	11	..
6	28	20	19	..
7	26	35	7	27

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† Present address: Department of Pharmacology; Smith, Kline & French Laboratories; Philadelphia, Pa.
‡ Associate professor of Pharmacology, Temple University School of Pharmacy, Philadelphia, Pa.

auriculo-ventricular junction. The dorsal ventricular wall was cut antero-posteriorly and then completely severed from the intestine which passes through the ventricle. After severing the remaining auricle, the heart was attached to a chamber containing 40 ml. of sea water at room temperature (24–30°) and aerated with air. The thread was attached to a heart lever and recordings were made on a slow, smoked-drum, Livingston kymograph.

The figures tabulated in the tables represent the responses (in per cent inhibition) to *l*-arterenol which were determined by measuring the lowest amplitude within three minutes after each administration of *l*-arterenol, and comparing it with the amplitude recorded immediately preceding the administration of the drug.

Demonstration of Tachyphylaxis on the Isolated *Venus* Heart.—Three or four 0.4-ml. doses of *l*-arterenol (Levophed Bitartrate, 0.1% base, Winthrop Laboratories) were administered every six minutes to the bathing fluid without washing the heart, so that concentrations of 1×10^{-6}, 2×10^{-6}, 3×10^{-6}, and 4×10^{-6} of *l*-arterenol (as the base) were successively attained. A lack of response to the third or fourth dose of *l*-arterenol (3×10^{-6} or 4×10^{-6}) was indicative of tachyphylaxis.

In 45 determinations, 24 and 10 hearts were unresponsive at concentrations of 3×10^{-6} and 4×10^{-6} respectively. The remaining 11 hearts either were not completely inhibited or were markedly depressed as indicated by a negative inotropic and chronotropic response (Table I).

With the initial concentration of 1×10^{-6} of *l*-arterenol, a transient inhibition of amplitude of contraction was noted. The duration of inhibition varied, but generally recovered within three minutes. A transient increase in frequency as well as an increase in tone were noted, both of which were generally diminished with subsequent doses. However, the magnitude of inhibition varied over a wide range with each animal.

Responses to *l*-arterenol may be classified into three types, with the majority falling into the first two categories. After recovery from a transient inhibition, the amplitude returns essentially to normal in the first type (Fig. 1); the amplitude becomes greater than normal in the second type (Fig. 2); the third type is characterized by the failure of the heart to recover from the initial inhibition, and with the subsequent administration of *l*-arterenol, a greater degree of inhibition occurs.

These variations in responses may be attributed in part to the variable size and freshness of the clams.

The Effect of Pretreatment with Ephedrine Sulfate on the Demonstration of Tachyphylaxis.—After a normal recording was taken, varying quantities (0.13, 0.27, 0.40, and 0.50 milliliter) of 1% ephedrine sulfate were added to the bath to attain final concentrations (as the salt) of 3×10^{-6}, 7×10^{-6}, 1×10^{-4}, and 1.2×10^{-4} respectively. After a four-minute interval, the heart was challenged with three or four 0.4-ml. administrations of *l*-arterenol.

In these pretreated hearts, the degree of inhibition after each administration of *l*-arterenol was diminished when compared to the inhibition produced by *l*-arterenol alone. Tachyphylaxis was demonstrated upon the third or fourth dose of *l*-arterenol

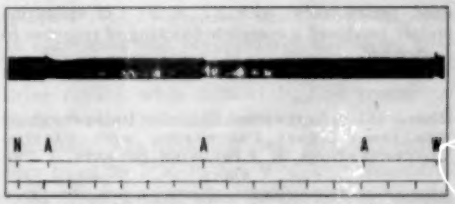

Fig. 1.—Demonstration of tachyphylaxis on the isolated *Venus mercenaria* heart with *l*-arterenol, Type I. Legend: N—normal contraction; A—successive bath concentrations of *l*-arterenol as follows: 1×10^{-6}, 2×10^{-6}, 3×10^{-6}; W—wash with fresh sea water. Time—in minutes.

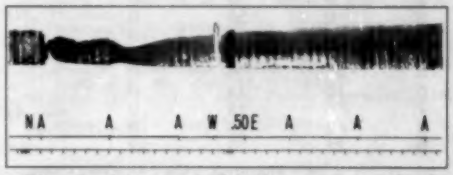

Fig. 2.—*l*-Arterenol-elicited responses of the *Venus* heart pretreated with ephedrine sulfate (1.2×10^{-4}). Legend: *N*—normal contraction; A—successive bath concentrations of *l*-arterenol: 1×10^{-6}, 2×10^{-6}, 3×10^{-6}, 1×10^{-5}, and 2×10^{-5}; W—wash with fresh sea water; .50 E—ephedrine sulfate concentration (1.2×10^{-4}); Time—in minutes.

Fig. 3.—*l*-Arterenol responses of the isolated *Venus* heart bathed in previously oxidized sea water. Legend: *N*—normal contraction; C—change to previously oxidized sea water; A—successive bath concentrations of *l*-Arterenol: 1×10^{-6}, 2×10^{-6}; W—wash with fresh sea water; Time—in minutes.

in hearts pretreated with 3×10^{-6} concentrations of ephedrine (5 determinations). The responses were varied in those pretreated with 7×10^{-6} concentrations (9 determinations) and 1×10^{-4} (16 determinations) concentrations of ephedrine sulfate, while with 1.2×10^{-4} (8 determinations), the response to *l*-arterenol was completely blocked (Fig. 2, Table II).

Although pretreatment with 3×10^{-6} of ephedrine sulfate had no effect on the production of tachyphylaxis, varied responses were noted in hearts pretreated with concentrations of 7×10^{-6} and 1×10^{-4} respectively. In eight cases, the heart was refractory to 1×10^{-6} of *l*-arterenol but responses occurred with higher concentrations; the heart was unresponsive to *l*-arterenol in five cases; tachyphylaxis was demonstrated in seven cases. How-

ever, pretreatment with 1.2×10^{-4} of ephedrine sulfate produced a complete blocking of response to *l*-arterenol.

TABLE II.—*l*-ARTERENOL-ELICITED RESPONSES[a] OF THE *Venus* HEART PRETREATED WITH VARYING CONCENTRATIONS OF EPHEDRINE SULFATE, 1 PER CENT

No. Detn.	3×10^{-5} Ephedrine, Then *l*-Arterenol Concn. 1×10^{-5}	2×10^{-5}	3×10^{-5}	4×10^{-5}
1	12	0	0	..
2	15	27	0	..
3	17	19	0	..
4	18	28	11	0
5	100	17	26	0
No. Detn.	7×10^{-5} **Ephedrine, Then *l*-Arterenol Concn.** 1×10^{-5}	2×10^{-5}	3×10^{-5}	4×10^{-5}
1	0	0	0	..
2	0	20	0	..
3	0	36	0	..
4	0	9	17	0
5	12	6	0	..
6	16	14	0	..
7	35	13	0	..
8	62	16	0	..
9	32	23	22	..
No. Detn.	1×10^{-4} **Ephedrine, Then *l*-Arterenol Concn.** 1×10^{-5}	2×10^{-5}	3×10^{-5}	4×10^{-5}
1	0	0	0	..
2	0	0	0	..
3	0	0	0	..
4	0	0	0	..
5	0	10	0	..
6	0	23	0	..
7	0	10	11	16
8	0	27	15	12
9	0	41	63	..
10	9	0	0	..
11	29	0	0	..
12	23	31	0	..
13	36	40	0	..
14	53	8	0	..
15	14	7	7	..
16	52	32	6	12
No. Detn.	1.2×10^{-4} **Ephedrine, Then *l*-Arterenol Concn.** 1×10^{-5}	2×10^{-5}	3×10^{-5}	4×10^{-5}
1	0	0	0	..
2	0	0	0	..
3	0	0	0	..
4	0	0	0	..
5	0	0	0	..
6	0	0	0	..
7	0	0	0	..
8	0	7	7	..

[a] Per cent inhibition of the preadministration amplitude after *l*-arterenol.

The Effect of Previous Oxidation on the Demonstration of Tachyphylaxis.—To determine the effect of previous oxidation of the bathing fluid on the demonstration of tachyphylaxis, a fresh heart was suspended in sea water in which tachyphylaxis had been previously demonstrated on a second heart. After recovery from the inhibition caused by this change of bathing fluid, the *Venus* heart was challenged with three 0.4-ml. administrations of *l*-arterenol.

Of the 22 determinations recorded, no responses were observed with 1×10^{-5} concentrations of *l*-arterenol in seven cases; in six cases, no response was observed with 2×10^{-5} of *l*-arterenol; in two cases, tachyphylaxis was not demonstrated with 3×10^{-5} concentrations of *l*-arterenol. In the remaining cases, the heart either failed to recover from the inhibition which occurred consistently with the change in bathing fluid or failed to recover to a sufficient degree to carry out the remaining procedures (Table III).

The normal heart is immediately arrested in systole upon changing to the bathing fluid in which tachyphylaxis had previously been demonstrated. During a variable time interval (averaging 7–9 minutes), the heart gradually relaxed until the contraction returned spontaneously, usually at a normal rate, and the amplitude progressively increased. When the contraction had attained a maximum, the heart became unresponsive to either the 1×10^{-5} or 2×10^{-5} concentration of *l*-arterenol (Fig. 3).

TABLE III.—*l*-ARTERENOL RESPONSES[a] OF THE ISOLATED *Venus* HEART BATHED IN PREVIOUSLY OXIDIZED SEA WATER

No. Detn.	Concn. of *l*-Arterenol 1×10^{-4}	2×10^{-4}	3×10^{-4}
1	0	0	0
2	0	0	0
3	0	0	..
3	0	0	..
4	0	0	..
5	0	0	..
6	0	..	..
7	0	..	..
8	7	0	..
9	8	0	..
10	9	0	..
11	31	0	..
12	31	0	..
13	21	0	8
14	27	9	7
15	17	36	40
16–22	Insufficient recovery or no recovery from inhibition.		

[a] Per cent inhibition of the preadministration amplitude following each 0.4 ml. dose of *l*-arterenol.

DISCUSSION

Throughout these experiments with *l*-arterenol, the pink discoloration referred to by Pless and Mann (6) was not observed, but by increasing the rate of aeration beyond the normal requirements, a pink discoloration was noted.

Although a discoloration was not observed in these experiments, the oxidation of *l*-arterenol must occur to some extent, due to the instability of *l*-arterenol in the presence of various chemical and physical factors which are present in this experimental procedure. However, since the inhibition caused by the change to the previously-oxidized solution resembles the response to a higher concentration of *l*-arterenol, this would indicate that the oxidation of *l*-arterenol had not occurred to any appreciable extent.

In speculation, regarding the basis for *l*-arterenol tachyphylaxis, Welsh (7) postulated that 5-hydroxytryptamine may be the normal excitatory mediator

of the *Venus* heart for the responsiveness of the heart occurs to extremely low concentrations of this agent. Ergotoxine ethane sulfonate (8) is also stimulatory, while lysergic acid diethylamide (9) specifically blocks the action of 5-hydroxytryptamine. Since all of these compounds have an indole configuration in common, perhaps the *l*-arterenol may be oxidized to some indole or indole-like configuration at the receptor sites. The greater sensitivity of the postulated *l*-arterenol oxidation product(s) may overcome the inhibitory action of *l*-arterenol and with the subsequent addition of the latter, no further inhibitory response can be exerted; thereby eliciting tachyphylaxis. The oxidation product(s) could be formed in the bathing fluid, but in view of the prolonged inhibition of rhythm which occurs with the change of bathing fluid, this seems unlikely according to this postulation.

SUMMARY

Although great variations in the response to repeated administrations of *l*-arterenol have been observed, tachyphylaxis was demonstrated in the isolated *Venus mercenaria* heart. The response to *l*-arterenol was progressively altered by pretreatment with increasing concentrations of ephedrine sulfate, while sufficiently high concentrations completely blocked the typical *l*-arterenol response. The fresh heart was generally refractory to either 1×10^{-6} or 2×10^{-6} concentrations of *l*-arterenol when suspended in bathing fluid in which tachyphylaxis had been previously demonstrated.

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Dextran Localization and Retention in Normal and X-Ray Irradiated Rabbits*

By LAWRENCE E. DETRICK, HARVEY C. UPHAM, DOROTHY HIGHBY, and THOMAS J. HALEY

Intravenous saline or dextran had no influence upon the effects produced by 500 r acute whole body x-irradiation in rabbits. The irradiation caused a post-irradiation shock-like syndrome in rabbits and minor histopathological changes in the liver. Neither saline nor dextran affected the fatal termination of the syndrome. In rabbits, 500 r x-irradiation was not a noxious stress of sufficient magnitude to impair metabolic or excretory functions of organs and alter dextran storage in tissues examined sixty days later. When examined sixty days post-irradiation, there was no significant difference in tissue storage or distribution of dextran between irradiated and nonirradiated animals. The intensity of dextran storage within rabbit organs was directly proportional to dosage and inversely proportional to the time after injection in those organs examined prior to the sixty day sacrifice interval. Irradiation did not affect storage either qualitatively or quantitatively.

A PARENTERAL SOLUTION of the polysaccharide dextran has been under intensive investigation both clinically (1 – 3) and experimentally (4 – 6), because of its potentiality as a safe, effective, and readily available plasma volume expander. Dextran has been recommended for the treatment of the mass casualties which might occur during a disaster (7). Information on the toxicity and tissue retention of intravenously injected dextran following acute whole body irradiation is lacking. Such information is necessary because any condition which damages the metabolic and excretory organs may lead to tissue storage of injected material (8). The present investigation has studied the toxicity, tissue distribution, and retention of intravenously

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injected dextran[1] solution in normal and irradiated rabbits.

EXPERIMENTAL

Female Dutch strain rabbits weighing 1.4 to 1.6 Kg. were used after implanting an indwelling catheter according to the method of Detrick and Rhodes (9). Groups of eight animals each were used for the single injection series; and groups of 12 animals each were used for the multiple injection series (see Table I for details). Each injection, saline or dextran, was of a total volume of 75-ml. given at a rate of 25 ml./hr. This clinical dextran contained 5–10% of material with average molecular weights as high as 200,000 and as low as 25,000, with the balance having an average molecular weight of 70,000. Necropsies were performed on all animals that died during the course of the experiments and on the survivors sixty days post-irradiation. These latter animals were subjected to a 72-hr. food deprivation period prior to sacrifice to reduce body glycogen stores and thus reduce false positive staining reactions. Tissue specimens (Table II) were removed, fixed in neutral formalin, and stained with hematoxylin and eosin. Contiguous slices of the above specimens were also fixed in cold absolute alcohol and serial tissue sections were differentially stained by the alcoholic periodic acid-aqueous Schiff's method for dextran (4). Five hundred r acute whole body x-irradiation was administered to the animals from above and below with two 250 KVP Picker Industrial Units operating simultaneously. Technical factors were: 250 KVP; FOD 55 cm.; filters—Cu 0.21 mm. inherent and 0.5 mm. parabolic and 1 mm. Al; size of field—whole body; rate—50.25 r/min. measured in air with a Victoreen Thimble r-meter.

TABLE I.—DEXTRAN INJECTION IN RABBITS

Group No.[a]	Post r Injection Time	No. of Injections	Solution Injected	Amount of Dextran ml.	Gm.
1	1 hr.	1	Dextran	75	4.5
2	1 day	1	Dextran	75	4.5
3	3 day	1	Dextran	75	4.5
4	7 day	1	Dextran	75	4.5
5	11 day	1	Dextran	75	4.5
6	1 hr.	1	Saline	75	0
7	3 day	1	Saline	75	0
8	0	0	Control	0	0
9	1 day[b]	1	Dextran	75	4.5
10	1 day[b]	1	Saline	75	0
11	1 day	9	Dextran	75	40.5
12	1 day	9	Saline	675	0
13	1 day[b]	9	Dextran	675	40.5
14	1 day[b]	9	Saline	675	0
15	0	0	Control	0	0

[a] Groups 1–10 contained 8 animals each. Groups 11–15 contained 12 animals each.
[b] Non-irradiated controls.

RESULTS

General Effects of Infusion and Irradiation.—After sixty days, the saline control groups (Table I) showed no changes which could be attributed to the saline infusions. However, the nonirradiated animals showed varying degrees of PAS staining which has been attributed to glycogen, basement membrane, mucin, colloid, and hyaline materials (1). Such naturally occurring materials were observed in all tissues examined and did not appear to increase following irradiation. The pre-sacrifice fasting of the animals appeared to decrease this staining reaction, thus increasing the probability that the remaining material found in the tissues of the dextran treated animals was actually dextran.

The 500 r whole body irradiation produced a 13% mortality in sixty days. Post-irradiation shock-like deaths (5–7 hours PR) were ushered in by a short period of restlessness, inactivity, fall in body temperature, lack of response to mild stimuli, and terminal convulsions. Intravenous injections of either dextran or saline solution administered during the development of the shock-like syndrome in no way influenced the eventual fatal outcome. Pneumonitis, uncomplicated by dextran injections, was the

TABLE II.—INTENSITY OF DEXTRAN STORAGE IN ORGANS OF IRRADIATED AND NONIRRADIATED RABBITS[a]

No. of Dextran Injections	Single[b]					Nine[b]		
Radiation	500 r				None	500 r		None
Survival in Days	Died 8	Died 15	Died 32	Sacrificed 60	Sacrificed 60	Died 19	Sacrificed 60	Sacrificed 60
Liver	4	3	2	0–3	1–2	5	4	4
Kidney	4	3	1	0–1	0–1	5	3	3–4
Adrenal	1	1	0	0	0–1	5	2–3	3–4
Spleen	3	1	0	0	0	5	2–3	3
Lymph node	...	...	...	0–1	0	4	2–3	2–3
Bone marrow	4	2	1	0–1	0–1	5	3–4	3
Lung	2	2	2	0–1	0	4	1	1
Pancreas	3	2	2	0–1	0–1	5	1–3	1–3
Small bowel	1	4	1	0–1	0–1	2	0–1	1
Large bowel	...	...	...	0–1	0–1	4	1–2	2
Muscle	2	1	1	0	0	3	1	1
Heart	3	3	1	1–2	0–1	4	1	2
No. of animals		10		30	8	1	11	12

[a] Intensity of dextran storage: 1—minimal; 2—slight; 3—moderate; 4—abundant; 5—intensive.
[b] Non-dextran injected groups omitted: Number of animals, single (S), multiple (M). Irradiated saline S (16), M (12); Nonirradiated saline S (8), M (12); irradiated S (8); M (11).

[1] The authors wish to thank Dr. Walter E. Ward of Cutter Laboratories for the clinical dextran solutions (Lot #58293-B) used.

most common cause of late irradiation deaths (8–32 days PR). Dextran injections did not cause severe reactions in either irradiated or nonirradiated animals. Normal rabbits had a body weight gain of 43% in fifty-seven days. Irradiated animals lost 6% of their initial body weight in three days, but were only 14% below that of the normal animals on the last day. Dextran infusions did not alter significantly the body weight curves of either group.

The identification of dextran storage in organs, as summarized in Table II, was made by the presence of purple to blue-black staining material in alcohol-fixed, alcohol-PAS stained tissue sections that were absent when similarly fixed sections were subjected to water and PAS staining. In Table II, the distribution of stored dextran has been designated for the organ as a whole.

The data in Table II show clearly that the intensity of dextran deposition in rabbit organs varies directly with the amount administered and inversely with the time post-injection. Dextran storage was the greatest in the organs of the multiple injected irradiated rabbit that died on the nineteenth PR day (eight days after the last injection). On PR day sixty, the amount of dextran in the organs of the multiple injected irradiated or non-irradiated animals was reduced, but by no means absent; and no significant difference existed in the dextran retention of the respective organs of either group.

Of the singly injected irradiated rabbits that died, the greatest dextran retention occurred in the organs of the eight-day death animal. Storage progressively decreased with time and was least in the organs on the last day (Table II); at that time more retention was seen in the liver, kidney, and heart than in the remainder of the organs. On day sixty, dextran storage in the organs of the singly injected irradiated and non-irradiated animals was approximately equal.

Histological Localization of Dextran After One Injection

The histopathology of the irradiated animal that died eight days post-irradiation (see Table II) was as follows:

Liver.—Abundant fine blue granules occurred in parenchymal cells. They were more prominent in the centrilobular areas and decreased toward the triads. Larger and more compact globular material filled the Kupffer cells. Free granules were not present within blood vessels, but the histiocytes in the adventitia were filled. Vacuolization of parenchymal cells was more prominent at the center and periphery of the lobule.

Kidneys.—There was a patchy distribution of blue granular material in the tubular cytoplasm that varied from fine granules within cells and the lumen to complete architectural obliteration of the tubules by solid dark blue material filling segments of varying length. This change involved only a small fraction of the total number of tubules. Glomeruli contained only an occasional blue staining phagocyte; lumens of blood vessels were free of granules but extensive retention occurred in histiocytes of the interlobular stroma, peri-pelvic fat, and beneath the pelvic mucosa. Tubular lumens throughout the kidney were filled with pink staining hyaline casts and a different mahogany colored," naturally occurring" PAS positive, water insoluble material was seen in the tubular cells of the neck of the glomerulus. Irradiation increased this latter material.

Adrenal.—The zona fasciculata cells of the adrenal contained diffusely scattered blue granules within the cytoplasm. Larger blue-black globules occurred in phagocytes beneath the capsule, peri-adrenal fat, and interstitial tissue of the cortex. No deposition was observed in cells of the zona glomerulosa, zona reticularis, or medulla.

Spleen.—There was a considerable reduction in size; follicles of the white pulp were small and the capsule, trabeculae, and hemosiderin deposits assumed exceptional prominence. Fine blue granules lined the sinusoids and filled the reticular cells of the red pulp. Retention was also observed in phagocytic cells of the hilar fat and capsule, and in the adventitia of blood vessels.

Reticular Bone Marrow Cells.—An abundance of dextran granules was seen uniformly distributed throughout this tissue. Vascular channels contained no dextran but some free granules were present within hemapoietic tissue.

Lung.—Localized PAS-positive, staining, phagocytic cells were seen, particularly at the periphery of inflammatory lesions, about lymphoid tissue, and within thickened alveolar walls. Minimal free granules were observed in the tissue. Histiocytes of the capsule and adventitia of blood vessels were also involved.

Pancreas.—Retention consisted of blue granules within reticulo-endothelial cells of the surrounding fat, in interlobular connective tissue, about blood vessels, and occasionally as free granules in acinar cells, but never within the cytoplasm of islet cells.

Small Intestine.—Extensive "natural PAS-positive staining material" occurred in Brunner's glands and epithelial tissue giving such a dark red to blue color that dextran, if present, could not be detected. Dextran storage was found in histiocytes of the serosal coat, between muscle layers, and in connective tissue about blood vessels of the submucosa and villi.

Muscle.—Striated muscle stored a slight amount, and cardiac muscle a moderate amount of dextran in phagocytic cells of connective tissue and about the blood vessels. No free granules occurred within the muscle fibers of either tissue.

Histological Localization of Dextran After Nine Injections

The larger amount of dextran produced greater tissue storage phenomena in organs examined eight days after the last injection, as characterized by the following:

Liver.—Dextran retention in the cytoplasm of parenchymal liver cells varied in size from globules to granular aggregates that were fairly evenly distributed from the central vein to the triads. Free granules were present in blood vessels and in the sinusoids. Kupffer cells were filled but not conspicuous because of the heavy deposition in liver cells. Parenchymal cells contained a greater residual glycogen content and the vacuolization was more apparent about the central veins and triads than in cells of the center of the lobule.

Kidney.—A heavier tissue deposition and a more generalized tissue involvement characterized storage phenomena within the kidney. Dextran was observed in all glomeruli varying in size from free granules to large masses of blue-black colored material. The number of localized solid blue tubular cells and casts was increased. Fine blue granules occurred in the majority of tubular cells and there was a much more prominent involvement of the interlobular reticulo-endothelial cells.

Adrenal.—Dextran deposition occurred in histiocytes of the surrounding fat, in and beneath the capsule. Cells of the zona glomerulosa and outer fasciculata were nearly solid blue, tapering off as discrete granular deposition in the inner fasciculata, reticularis, and medullary zones. Interstitial histiocytes of the entire organ were involved.

Spleen.—The spleen showed less shrinkage, a characteristic of recovery from irradiation. The follicles were larger and the capsule, trabeculae, and hemosiderin deposits less prominent. There was massive infiltration of free dextran granules in the red pulp. The white pulp was clear except for an occasional filled histiocyte in or about blood vessels. Free granules lined the sinusoids.

Lymph Nodes.—Only a delicate deposit of fine granular dextran was seen within reticulum cells and lining the sinusoids.

Bone Marrow.—Free dextran granules lightly infiltrated the marrow tissue and were present within blood vessels and filled the phagocytes.

Pancreas.—Dextran was stored as massive blue, triangular, extracellular infiltrations of free granules within interlobular connective tissues and as a diffuse granular infiltration in the cytoplasm of acinar cells, but not in the islet cells. The macrophages were filled and free granules were present within blood vessels.

Small and Large Bowel.—Blue staining material in histiocytes of the serosal coat, between muscle layers, and about blood vessels of the lamina propria, villi, and intestinal folds was observed.

Striated Muscle.—Storage did not occur within muscle fibers, but free granules were present in the connective tissue. Histiocytes in the connective tissue and about the blood vessels stained dark blue.

Cardiac Muscle.—In contrast with striated muscle, cardiac muscle did show a profuse infiltration of free dextran granules within the cytoplasm of fibers, but otherwise the storage distribution was the same.

DISCUSSION

Our findings agree with observations of others who found parenteral dextran a safe plasma volume expander for rabbits (6, 10). Single or multiple intravenous injections were uneventful; infiltration of free granules into the tissues, storage in parenchymal or phagocytic cells incited no histological reactions; observations to the countrary notwithstanding (11). An infusion of dextran was a safe, although ineffective, therapeutic procedure even when given during the developmental period of the irreversible post-irradiation shock. These findings indicate that dextran is much safer than dextrin, oxypolygelatin or PVP under the same experimental conditions (12–15).

Dextran tissue retention has been detected by histochemical, serological, and chemical methods (4, 6, 11). The alcoholic periodic acid-aqueous differential staining procedure was used successfully in dextran identification in the human (1), mouse (4), dog (8), and rabbit (16). Normal tissues contain no PAS-positive, water soluble material, but do show "naturally occurring" water insoluble materials such as glycogen, basement membrane, hyaline, colloid, and mucin (1). We observed similar staining reactions in control animals. Such staining reactions were not increased by irradiation. Irradiated dextran injected rabbits that died early showed abundant red hyaline casts throughout the kidney. Similar red casts have been reported in kidney tissue of both battle casualties (1) and burned mice (17). Irradiated splenic tissue also showed PAS-positive staining cells, but a generalized plasma cell cytosis was not seen in the rabbits. However, aggregates of PAS-positive plasma cells have been observed in rabbit spleens following stress of hyperimmunization (18), and plasma cell cytosis was a consistant finding in atomic bomb casualty tissues (19).

Intravenously injected dextran produced retention in parenchymal cells of the liver, kidney, adrenal and pancreas, and in reticulo-endothelial cells of all organs examined including the spleen, lymph nodes, bone marrow, lung, bowel, and muscle. The intensity of deposition was proportional to the amount given and inversely proportional to the post-injection time at which the tissues were examined. Dextran solutions were not tissue irritants in moderate amounts. The above findings agree with observations of others (1–6, 17, 20). Bull, *et al.* (6), pointed out that the lower molecular weight fractions of dextran were rapidly excreted whereas the higher fractions were retained in the body. Thus our observations of tissue deposition are probably related to retention of the higher molecular weight fractions in the dextran we used. Gray (21) using C^{14}-labeled dextran showed that eventually even the retained fraction is eliminated from the body. The observation of decreased tissue retention (Table II) confirms Gray (21).

The irradiation, itself, produced minor changes in the liver, kidney, bone marrow, spleen, lymphoid tissue, and lung. This agrees with the observations of Hagen, *et al.* (22).

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The Ontogenesis of Gramine in Barley Seedlings*

By V. E. TYLER, Jr.

A sensitive paper chromatographic technique was developed for the separation and identification of the basic nitrogenous compounds in barley seedlings. This method was utilized to follow the ontogenesis of gramine in normal and etiolated plants. It was established that gramine does not exist in the ungerminated seed, the coleoptiles, or roots of Charlottetown No. 80 barley, but is present in the leaf on the third day following germination and remains there in detectable quantities for at least fifty days. No qualitative differences in gramine content were observed between the normal and etiolated seedlings. The significance of these results with reference to seemingly conflicting previous reports is discussed.

GRAMINE, AN ALKALOID isolated from certain varieties of barley, *Hordeum vulgare* L., and also from an Asiatic reed, *Arundo donax* L., has been characterized chemically as 3-(dimethylaminomethyl)-indole (1). Employing a photometric method of determination, Euler, *et al.* (2, 3), established that of 18 varieties of barley seedlings investigated, five contained gramine. In these plants the alkaloid was not present in the coleoptile, but was detected in the roots and leaves, increasing in concentration in the latter organs from the base to the apex. It was further established that the total gramine content of the plant remains constant from the fourth to the tenth day following germination, but no alkaloid could be detected after one month.

More recently, Marion, *et al.*, in studies on the biogenesis of alkaloids, fed tryptophan labeled with C^{14} in the β position to germinating barley seedlings (variety Charlottetown No. 80) and were able to isolate radioactive gramine from the leaves, indicating that tryptophan serves as a precursor to gramine (4). Radioautographs of such barley leaves indicated that the tryptophan was first concentrated near the base of the leaves and then reappeared as gramine in the leaf tips (5). A further study (6), employing a mixture of tryptophans labeled in the 2 and β positions, verified that the conversion of tryptophan to gramine takes place without cleavage of the indole-alanine linkage.

Because gramine is a derivative of indole, a structure which is of considerable physiological interest, it appeared worth while to obtain more detailed information regarding the ontogenesis of the alkaloid, especially with respect to its reported occurrence in the root as well as its ultimate disappearance from the leaf.

EXPERIMENTAL

Chromatographic Identification of Gramine.—Before proceeding with the ontogenetic phase of the investigation, it was necessary to develop a sensitive process for the separation and identification of the basic nitrogenous compounds occurring in barley seedlings; specifically choline, gramine, and hordenine. The paper chromatographic method appeared well suited for this purpose, but gramine and hordenine proved to be extremely difficult to separate completely, and a large number of the more conventional organic solvent systems were employed without success. Good resolution was finally achieved using an aqueous solution of an electrolyte as suggested by Resplandy (7).

Mixtures of the bases were spotted on 1 × 22.5-in. strips of Whatman No. 1 filter paper and subjected to ascending formation with a 10% aqueous solution of ammonium sulfate for about five hours. A more rapid and equally satisfactory separation was achieved employing the circular formation method

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of Rutter (8). With Whatman No. 1 paper in a 15-cm. Petri dish, time of development was reduced to less than two hours. A particular advantage of this wash liquid was its aqueous character which made it possible to spot comparatively crude extracts of plant material on the chromatogram since many impurities (lipids, chlorophyll, etc.) are insoluble in it and do not move from their original position.

Localization of the bases was carried out by dipping the chromatogram in Munier's reagent (9). Average R_f values obtained by the ascending technique were: gramine 0.64, hordenine 0.78, and choline ran with the solvent front. Corresponding values were obtained by the circular method, but here standard substances were employed for reference purposes.

The Detection of Gramine in Seedlings.—Quantities of Charlottetown No. 80 barley seed[1] were germinated in moist air at room temperature as described by Meyer and Anderson (10). In cases where seedlings in an advanced stage of development (greater than ten days) were desired, the seeds were planted in soil contained in pots and allowed to develop under greenhouse conditions.

Two series of experiments were carried out. In the first, seedlings were grown in total darkness for periods of three, six, and eight days. When harvested, the plants were separated into root, coleoptile, and leaf and dried in a circulating air oven at 45°. Seedlings of the second group were allowed to develop subject to the normal diurnal variations of light and then treated as previously described. In this series, however, the period of development was extended to include the roots and leaves of plants harvested 10, 15, 20, 25, 30, 40, and 50 days after germination. A quantity of ungerminated seed was also tested for gramine, since the presence or absence of the alkaloid in these parts had not been established.

After drying, the various plant parts were ground to a No. 40 powder in a Wiley laboratory mill, weighed, and shaken in stoppered test tubes containing 1 ml. of ethanol per 100 mg. of plant material for one hour. The sample size employed generally ranged from 100 to 500 mg. The tubes were then centrifuged, the extract decanted into a small evaporating dish and reduced to dryness in a stream of warm air. The residue was then redissolved in 1 ml. of ethanol, and a quantity of this extract representing a minimum of 25 mg. of the dried plant material was spotted in small, successive portions on the chromatographic sheet or strip which was then treated as previously described. In this way, 5 μg. of gramine could be detected with certainty. Expressed in terms of the minimum quantity of extract spotted, this is equivalent to 0.02% of the dry weight of the plant material tested.

RESULTS AND DISCUSSION

Gramine could not be detected in the ungerminated seed or in the coleoptiles of either normal or etiolated seedlings tested three, six, and eight days following germination. In the leaves the alkaloid was present in both normal and etiolated plants three days after germination, which was the earliest stage of development at which it was possible to effect an efficient separation of the leaf and coleoptile. It continued to be present in the leaves of both series of plants until the experiment was concluded, in the case of the etiolated plants after eight days, in the case of the normal plants, fifty days. Gramine was never detected in the roots of any of the seedlings during the entire course of the experiment.

The discrepancies in these results in comparison to those reported by previous investigators may be explained on the basis of the use of different plant material or a difference in the methods employed. The procedure of Brandt, *et al.* (3), for estimating gramine involves an absorbance measurement at 280 mμ of a purified alkaloidal extract. In the case of roots tested in this way, the extract would also contain hordenine which absorbs strongly at this wavelength. This may account for the earlier report of gramine in the roots of barley seedlings.

Although there is a possibility that the distribution of gramine as well as its occurrence in the plant is a genetic variable which may differ with different varieties of barley, this hypothesis appears remote in the light of present knowledge. However, this same explanation could account for the existence of gramine in Charlottetown No. 80 barley leaves for at least fifty days following germination, although previous investigators were unable to detect it in month-old plants.

Failure to detect any gramine in the root, coupled with the reports of Marion, *et al.*, regarding synthesis during the initial growth of the leaf, seems to indicate conclusively that the latter organ is the exclusive site of synthesis, as well as storage, of gramine in Charlottetown No. 80 barley. This observation is particularly interesting in view of the numerous observations that the root is the "Hauptbildungsstätte" of many of the alkaloids (11). Although there are exceptions to this rule, such as the shoot synthesis of steroid alkaloids of the solanidine type and anabasine, such examples are not numerous. As Mothes points out, however, it is not too presumptuous to think that the present opinion concerning the locus of alkaloid formation is too limited, being based largely upon sporadic data. In any event, gramine may be added to the comparatively small group of alkaloids which are known to be synthesized and stored in the plant shoot.

[1] Supplied through the courtesy of R. B. MacLaren, Technical Officer, Experimental Farm, Charlottetown, Prince Edward Island.

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A Procedure for the Isolation and Determination of Trasentine in Solutions*

By ANTHONY J. TARASZKA and ARNOLD D. MARCUS†

Aqueous solutions of Trasentine® hydrochloride (β-diethylaminoethyl diphenylacetate hydrochloride) may be analyzed for residual concentrations of the intact ester by means of partition chromatography and subsequent nonaqueous titration. Aliquots of the aqueous solutions are introduced onto the silicic acid columns, prepared to contain an internal phase buffered to pH 4.8, as slurries with carbon tetrachloride and silicic acid. The Trasentine base is then selectively eluted with chloroform and the concentration of base determined by titration with standard perchloric acid in glacial acetic acid using α-naphtholbenzein indicator. The results obtained by this method are in good agreement with those obtained by other methods. In addition, the procedure described has a number of advantages for use in conjunction with kinetic studies.

PRIOR TO UNDERTAKING a study of the kinetics of hydrolysis of Trasentine hydrochloride (β-diethylaminoethyl diphenylacetate hydrochloride) it was necessary to choose or develop an analytical procedure which would permit accurate and precise dererminations of residual quantities of the intact drug. Although at least three ordinarily acceptable methods were already available, each of these had one or more inherent disadvantages which rendered it unsuitable for our needs.

The gravimetric method (1), based upon formation of the picrate, was entirely too time-consuming. Direct spectrophotometric determination (2) could not be employed because of the similarity in absorption spectra between Trasentine and diphenylacetic acid, one of the major degradation products. The procedure of Goddu, *et al.*, for esters (3) was considered, but the nature of the reagents required seemed to introduce the possibility of degradation other than that desired. It appeared, therefore, most plausible to develop a new method.

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† Present address: Merck Sharp and Dohme Research Laboratories, West Point, Pa.
The authors wish to thank Mr. Jack Cooper of Ciba Pharmaceutical Products, Inc., for his generosity in supplying us with the Trasentine hydrochloride and for making available to us his data relative to analytical procedures and general stability. They also wish to express their appreciation to Mr. Thomas Medwick of the University of Wisconsin, School of Pharmacy, for his valuable suggestion concerning the choice of indicators.

The present communication indicates the feasibility of employing partition chromatographic separation of the undegraded Trasentine base and subsequent titration of this base with perchloric acid in glacial acetic acid in the presence of a suitable indicator.

DISCUSSION OF THE EXPERIMENTAL APPROACH

Nonaqueous determination of Trasentine as the free base requires that the ester be separated completely from any β-diethylaminoethanol which might result from hydrolysis. This separation is vital inasmuch as both compounds respond as bases in the nonaqueous solvents suitable for elution of Trasentine. Although it would be desirable to effect a similar separation of Trasentine from diphenylacetic acid, this preference is a matter of elegance rather than necessity. The acid does not, as will be shown later, interfere with the titrations.

Because β-diethylaminoethanol is a much stronger base than Trasentine and much more soluble in water, it was assumed that silicic acid columns buffered at pH 6.0 would permit a simple separation of the two amines. In light of the pKa of diphenylacetic acid (*ca.* 4) it also seemed possible to retain the acid on the column while eluting the undegraded ester with chloroform. The flowing chromatogram shown in Fig. 1, which was obtained in the presence of β-diethylaminoethanol, apparently confirmed the suitability of such a procedure. When, however, this method was applied, the precision and accuracy were so poor as to necessitate some change.

The poor results seemed best attributable to either or both of two reasons. Either the Trasentine was being held on the column because of the adsorptive properties of silicic acid, or the ester was undergoing degradation during the course of separation. The first of these alternatives was regarded as unlikely in view of a lack of any significant tailing in the chromatogram. Because it is known (1) that the rate of hydrolysis of Trasentine increases markedly with hydroxyl ion concentration, the second alternative was pursued. Since Trasentine exhibits greatest stability between pH 4–5, the columns were changed to provide an internal phase buffered to pH 4.8. This simple change permitted an excellent order of precision and accuracy without any noticeable alteration in the shape of the chromatogram.

EXPERIMENTAL

Materials.—Trasentine hydrochloride; silicic acid, Mallinckrodt, Chromatography Grade; 0.5 *M* pH 4.8 acetate buffer; chloroform, Baker analyzed reagent; carbon tetrachloride, N. F.; α-naphthol-

benzein, 0.1% in glacial acetic acid; 0.01 *N* perchloric acid in glacial acetic acid.

Equipment.—Chromatographic columns, 2 (i.d.) x 40 cm. provided with suitable delivery tubes and indented at the base so as to hold glass wool; close fitting glass plungers for packing the columns; 5-and 10-ml. burets.

Procedure.—Pack the columns according to the procedure of Higuchi and Patel (4). Use 20 grams of silicic acid as the holding phase, 20 ml. of 0.5 *M* pH 4.8 acetate buffer as the internal phase, and carbon tetrachloride as the external phase.

Pipet an exact volume (not to exceed 5.00 ml.) of an aqueous solution of Trasentine, adjusted to pH 4.8 and containing between 0.03 and 0.05 Gm. of the drug in each ml. onto a quantity of dry silicic acid numerically equal in grams to the volume of solution used. Mix thoroughly with a small spatula, add 10 ml. of carbon tetrachloride and form a slurry by thorough mixing. Transfer the slurry to the column using quantitative technique and pack it tightly onto the top of the column. When removing the plunger from the column, use care to avoid carrying along any adhering slurry. Rinse the container (preferably a 30-ml. beaker) with an additional 10 ml. of carbon tetrachloride and add the rinsings to the column. When all but the last traces of supernatant liquid have entered the column, force any of the slurry, which may have adhered to the sides of the column, downward onto the main portion. At this point, add a slurry prepared from 1 Gm. silicic acid, 1 ml. buffer, and 5 ml. of carbon tetrachloride to the column and pack down tightly.

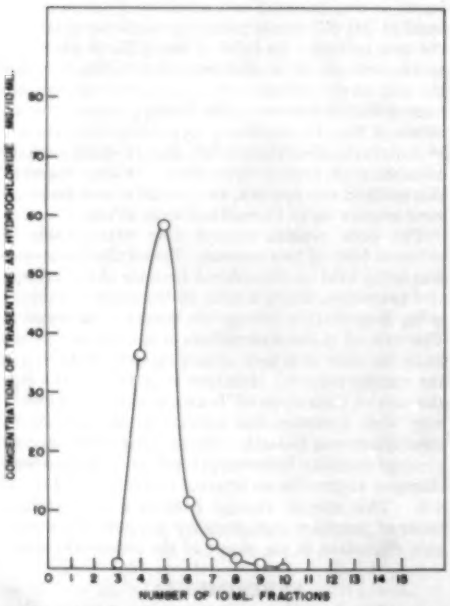

Fig. 1.—Partition chromatogram showing the selective elution of Transentine from a buffered silicic acid column. In addition to 120 mg. of Trasentine, the sample also contained an equivalent amount of 2-diethylaminoethanol.

When all of the supernatant liquid has again entered the body of the column, pipet exactly 5 ml. of chloroform into the column and begin collecting the eluate in a 100-ml. volumetric flask. Allow all but a bare trace of the chloroform to drain into the column, add a second 5-ml. portion of chloroform and allow it, too, to drain into the column. Chloroform may then be added in larger amounts but the flow rate should not exceed 2 ml. in three minutes.

After 100 ml. of eluate have been collected in the volumetric flask, take 10-ml. aliquots for determination of the Trasentine content. To each 10-ml. aliquot add 5 drops of 0.1% α-naphtholbenzein in glacial acetic acid and titrate with 0.01 *N* perchloric acid in glacial acetic acid to the proper visual end point. Perform a blank to compensate for the basicity of the indicator.

Each ml. of 0.01 *N* perchloric acid used is equal to 3.479 mg. Trasentine hydrochloride.

RESULTS

As shown in Table I, the analytical procedure described above permits quantitative recovery of Trasentine from aqueous solutions.

TABLE I.—RECOVERY OF TRASENTINE FROM AQUEOUS SOLUTIONS

Sample No.	Trasentine HCl Added, mg.	Found, mg.	Recovery, %
1	123.9	123.5	99.7
	123.9	124.6	100.5
	123.9	123.3	99.5
2	116.1	116.2	100.0
	116.1	116.9	100.7
	116.1	116.8	100.7
3	118.4	117.2	99.1
	118.4	117.3	99.1
	118.4	117.5	99.2
4	138.7	138.1	99.6
	138.7	137.4	99.1
	138.7	137.4	99.1

It was stated previously that diphenylacetic acid, even if it were eluted along with the Trasentine base, would not interfere with the titrations. The data in Table II testify to the validity of this assumption. These data were obtained by including an amount of diphenylacetic acid equivalent to that which would be present if all of the Trasentine had hydrolyzed. Since the acid is not completely soluble at pH 4.8, the undissolved acid was allowed to remain in suspension. The suspensions were shaken thoroughly prior to each withdrawal of a sample for

TABLE II.—RECOVERY OF TRASENTINE HYDROCHLORIDE IN PRESENCE OF DIPHENYLACETIC ACID

Sample No.	Trasentine HCl Added, mg.	Found, mg.	Recovery, %
1	118.9	118.3	99.5
	118.9	118.8	99.9
	118.9	118.0	99.2
2	138.5	137.2	99.1
	138.5	137.5	99.3
	138.5	137.9	99.5

analysis. Although this may have detracted somewhat from the accuracy of the method, the essential point is, nevertheless, clearly demonstrated.

SUMMARY AND CONCLUSIONS

1. The concentration of Trasentine hydrochloride in aqueous solutions may be determined by selectively eluting the free Trasentine base from partition chromatographic columns prepared from silicic acid, 0.5 *M* pH 4.8 acetate buffer and carbon tetrachloride. Chloroform is used as the eluant. One of the products of hydrolysis of Trasentine, β-diethylaminoethanol remains on the column. While it is not certain whether the other product, diphenylacetic acid remains behind, this is of little consequence inasmuch as the acid does not interfere with determination of Trasentine base by nonaqueous titration.

2. The procedure described is apparently precise to within 1% and offers advantages over other methods for use in conjunction with kinetic studies.

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A Study of the Decomposition of Glucose Solutions*

By NORVAL E. WEBB, Jr.,† GLEN J. SPERANDIO, and ALFRED N. MARTIN

Glucose solutions were thermally decomposed under varying conditions. Preliminary findings showed that the formation of 5-hydroxymethylfurfural from glucose was subject to general acid-base catalysis. Results also indicate that the rate of reaction was inversely proportional to the initial concentration of glucose.

THE INSTABILITY OF GLUCOSE SOLUTIONS is one of the major problems encountered in the field of parenteral medication. Although considerable work is reported in the literature concerning this problem, little seems to have been accomplished in prolonging the shelf life of glucose injections.

Investigators have studied the effects of heat on glucose solutions (1–4), since autoclaving is the most practical method for the sterilization of parenteral products. It has been shown that an excessive amount of heat causes the formation of a yellow color, and the theory has been advanced that at least part of this color is due to the polymerization of 5-hydroxymethylfurfural (hereafter referred to as 5-HMF) (3, 5), a decomposition product of glucose. Singh, *et al.* (6), found that the extent of coloration of glucose solutions paralleled the extent of 5-HMF formation, the least amount of coloration occurring at pH 3.0. The rate of formation of this product has been assumed to be proportional to the decomposition rate of glucose (7), which would lead to the conclusion that the rate of glucose degradation in solutions could be determined, under varying conditions, by measuring the rate of formation of 5-HMF.

Brönsted and Guggenheim (8) found a general acid-base catalysis in the mutarotation of α-glucose and attributed it to the anions and corresponding undissociated acids. It would be reasonable to assume that the decomposition of glucose to 5-HMF would also be subject to general acid-base catalysis.

It was the purpose of this investigation to determine the effect of varying concentrations of glucose in solution upon the rate of decomposition, to study the effect of different buffering systems as a means of establishing general acid-base catalytic effects, and to prepare an Arrhenius plot to determine the energy of activation for the conversion of glucose to 5-HMF.

EXPERIMENTAL

Materials.—Mallinckrodt A. R. anhydrous dextrose was chosen for use in this study. Redistilled water was used in the preparation of all solutions. The 5-HMF used in preparing the standard spectro-

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† Assistant Professor of Pharmacy, South Dakota State College of Agriculture and Mechanic Arts, Brookings.

photometric curve was obtained[1] as a dark brown viscous liquid and was crystallized and purified by vacuum distillation at 114–117° at a pressure of 1 mm. of mercury. All decomposition runs were carried out using 100-ml., Army type, serum bottles of type-I glass.

Apparatus.—All pH determinations were made with a Beckman model H2 glass electrode pH meter. The Beckman DU spectrophotometer with ultraviolet attachment was used for the assay of 5-HMF, using 10-mm. fused silica cells. The glucose solutions were decomposed in a constant temperature oven, which could be controlled within a temperature range of ±0.5°.

Assay.—The wavelength of maximum absorbance for a solution of 5-HMF was found experimentally to be 284 mμ. A series of dilutions of 5-HMF in 10% glucose solution was accurately prepared, and the absorbances were determined at 284 mμ with a distilled water blank. The data were found to follow Beer's law over the range of 0.1 to 1.0. Absorbance was plotted against concentration and a line was fitted to the points by the method of least squares. In order to arrive at specific rate constants, the assay values were converted to mcg. of 5-HMF per mole of anhydrous glucose.

Methods.—With the exception of two runs made for the Arrhenius plot, all decomposition runs were conducted at a temperature of 100°. The solution containers were placed in the oven and attached to a sample-removal device which eliminated opening the oven to obtain the samples. Samples were taken initially and every thirty minutes for a period of two hours.

At the end of each decomposition run the samples were assayed for 5-HMF. After converting the absorbances of these samples to mcg. of 5-HMF per mole of glucose, the concentration of 5-HMF was plotted against time on rectangular coordinate graph paper. In a majority of cases the results were not linear; that is, the rate of formation increased with time, indicating what was considered to be autocatalysis. A typical plot is shown in Fig. 1. Specific reaction rates were determined by drawing a tangent to the origin of the curve by a method similar to that described by Lotshaw (9). The specific rate constant was expressed as k_i = (mcg. 5-HMF per ml./moles of glucose initially present per ml.)/hr., where the subscript (i) is used to indicate that the rate was determined from the initial part of the curve.

Concentration Effect upon Decomposition Rate.—A series of glucose solutions varying in concentration from 5 to 25% was decomposed under identical conditions and the specific reaction rates were compared. The solutions were maintained at a pH of 5.3 by the use of an acetate buffer system. The results are shown in Table I. It is seen that as the concentration of glucose increased the rate constant decreased. It is of interest to note that Zheltukhin (10) reported no effect on the rate of decomposition by the concentration of glucose with studies conducted at 145° and 167°; however, at 187° an increase was reported in the rate of decomposition with increased glucose concentration.

[1] Purchased from the Bios Laboratories, 17 W. 60th Street, New York 23, N. Y.

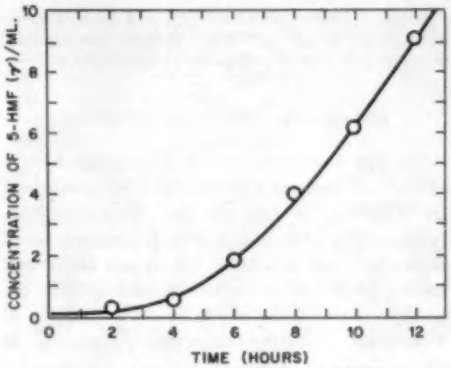

Fig. 1.—The rate of formation of 5-HMF from a 10 per cent anhydrous glucose solution at 100° C.

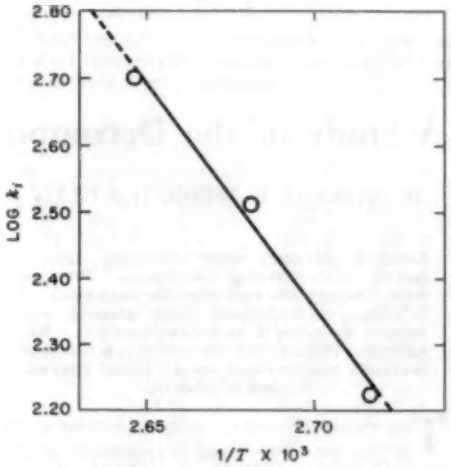

Fig. 2.—The effect of temperature upon the rate of glucose decomposition.

Since the rate of glucose decomposition decreased with the increase in glucose concentration under the conditions used in this study, it would be conceivable that by increasing the concentration of glucose in a buffered solution, and carrying out sterilization at a temperature of about 100°, the stability of glucose solutions for injection could be improved.

General Acid-Base Catalytic Effect.—To determine the acid-base catalytic effect upon the conversion of glucose to 5-HMF, buffered solutions containing 10% glucose were decomposed in the manner

TABLE I.—REACTION RATE CONSTANTS FOR VARYING CONCENTRATIONS OF GLUCOSE

Glucose, %	Mcg. 5-HMF/mole of Glucose/Hr. (10^{-3} k_i)
5	11.0
10	11.0
15	7.0
20	6.5
25	5.4

previously described. In no case was there any appreciable variation in initial and final pH. Three buffer systems were selected to cover a pH range of from pH 3.0 to pH 6.7. A formic acid-sodium formate system was used at pH 3.0, an acetic acid-sodium acetate system at pH 5.0, and a monobasic potassium phosphate-sodium hydroxide system at pH 6.7. The buffer ion concentration of the buffer system at each pH was varied. The results are shown in Tables II, III, and IV.

TABLE II.—DECOMPOSITION OF 10% ANHYDROUS GLUCOSE IN A FORMATE BUFFER SYSTEM AT A PH OF 3.0

Molarity of Total Formate Including Undissociated Acid	Mcg. 5-HMF/mole of Glucose/Hr. (10^{-4} k_0)
0.006	2.2
0.012	2.5
0.107	3.2
0.193	5.0

TABLE III.—DECOMPOSITION OF 10% ANHYDROUS GLUCOSE IN AN ACETATE BUFFER SYSTEM AT A PH OF 5.0

Molarity of Total Acetate Including Undissociated Acid	Mcg. 5-HMF/mole of Glucose/Hr. (10^{-4} k_0)
0.005	1.2
0.010	1.6
0.052	5.6
0.098	9.3
0.213	29.0

TABLE IV.—DECOMPOSITION OF 10% ANHYDROUS GLUCOSE IN A PHOSPHATE BUFFER SYSTEM AT A PH OF 6.7

Molarity of Phosphate Ion	Mcg. 5-HMF/mole of Glucose/Hr. (10^{-4} k_0)
0.050	3.0
0.100	3.4
0.200	3.8

In each case it can be seen that as the molar concentration of the buffer increased the rate of decomposition was accelerated. It may be concluded from this data that the conversion of glucose to 5-HMF is subject to general acid-base catalysis. These findings indicated that the catalytic effect of anions and undissociated acids of any buffer system that one might wish to employ in the preparation of glucose solutions, must be considered as well as the hydrogen ion concentration of the solution.

Temperature Effect.—For this portion of the study 10% glucose solutions were decomposed at three different temperatures: 95, 100, and 105° ± 0.5°. The solutions for decomposition were prepared using a sufficient quantity of hydrochloric acid to give a normality of 0.001. Under these conditions the pH of the solutions remained unchanged throughout the duration of the runs. The results are shown in Fig. 2. The line best fitting the points was determined visually and the slope of the line was calculated to be -6.7×10^3. From the classical Arrhenius equation and the value obtained for the slope in Fig. 2, the activation energy was calculated to be roughly 31,000 calories. Although only three runs were made at 5-degree intervals the activation energy agreed well with the average value of 32,800 calories reported by Saeman (11).

It is not possible at this stage of the investigation to employ the decomposition data at elevated temperature for predicting the stability of glucose solutions at room temperature. The results obtained in preliminary experiments at temperatures below 100° were erratic and the autocatalysis ruled out the possibility of obtaining a specific reaction rate that would remain constant over extended periods of time.

SUMMARY AND CONCLUSIONS

1. The rate of formation of 5-HMF was used to obtain information concerning the effect of various conditions upon the rate of decomposition of glucose solution exposed to high temperatures.

2. The results at 100° showed an inverse relationship between the rate of decomposition and the concentration of glucose. This would indicate that perhaps greater stability of the solution could be achieved by increasing the glucose concentration.

3. The conversion of glucose to 5-HMF is subject to general acid-base catalysis as indicated by the increase in the rate of decomposition with increasing concentrations of buffer ions when hydrogen ion concentration is held constant. Therefore, the catalytic effect of ions other than hydrogen must also be considered when preparing buffered glucose solutions.

4. The energy of activation for the conversion of glucose to 5-HMF was found to be approximately 31,000 calories.

5. Further studies on autocatalysis and solvent effects in the conversion of glucose to 5-HMF are underway and will be reported at a later time.

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Evaluation of Suspensions Using Electrokinetic Measurements*

By G. L. STANKO† and H. G. DeKAY

The need for methods of evaluating and predicting the stability of suspensions has been recognized. The factors of pH and viscosity do not enable the investigator to predict and evaluate a series of suspensions. Electrokinetic factors appear to be of value as these measurements seem to indicate the stability of a suspension.

NINETY per cent of the drugs used at the present time did not exist ten years ago (1). A great majority of these new drugs are synthetic chemicals possessing characteristics that often make it difficult to formulate a stable suspension. There is also a wide choice of surface active agents, emulsifying agents, flavoring agents, suspending agents, and preservatives that increases the compounding problem. Therefore, with the large number of potential formulas to be considered, a need arises for screening measurements to indicate the stability of the product. These measurements would make it possible to determine more rapidly and discard the unstable formulas early in the formulation study.

The methods generally used for the evaluation of a pharmaceutical suspension have been measurement of viscosity (2), pH (3), sedimentation (4), and particle size (5). Other approaches to the problem have included the use of a shaking machine (6) and a sedimentation-kymograph (7). These studies indicate the suspending ability of a substance for a drug, but do not predict whether agglomeration or caking will occur. In nearly all suspensions, some sedimentation will take place, but the important aspect is whether the sediment can be easily and uniformly resuspended. Thus, there seems to be a need for a method of more quantitatively determining the optimum pH, suspending, and wetting agents so that the most compatible formula can be obtained.

A few reports in the literature have contained references to the electrostatic properties of a suspension (8–11). Incompatibilities were thought to be due to the opposite charges of ions and suspending agent. This aspect of pharmaceutical suspensions has not been very intensely investigated, although it has indications of being an important factor in determining the stability of a suspension.

The investigation of the stability of carbon in soap solutions resulted in the conclusion that zeta potential was a principal factor in producing a stable suspension (12). The effect of different electrolytes on a vanadium pentoxide sol and a chromium hydroxide sol was measured. When the electrokinetic potential fell below a certain point, the sols became unstable (13). The suspendability of various detergents for a manganese dioxide sol was studied. It was concluded that the magnitude of the zeta potential on the particles was the dominant variable in determining whether or not a stable suspension was obtained (14). In these studies of nonpharmaceutical suspensions, the electrokinetic factors have been shown to be of fundamental importance; therefore, it is the purpose of this paper to consider electrokinetic factors as a method of evaluating and predicting the stability of a pharmaceutical suspension.

EXPERIMENTAL

Suspensions of sulfamerazine in 5% concentration were prepared with the following suspending agents: methylcellulose, 4000 c. p. s. and 25 c. p. s; sodium carboxymethylcellulose medium and high viscosities; and sodium alginate. The suspending agents were used in 1% concentrations and all were preserved with 0.1% methyl parahydroxybenzoate. Each suspension was prepared with a mortar and pestle, then passed through a hand homogenizer. One hundred milliliters of each suspension was placed in a graduated cylinder for sedimentation observations, and the balance divided into four-ounce prescription bottles. These portions were used for the various periodic measurements in order to have an undisturbed sample. All suspensions were stored at room temperature on the laboratory desk.

Each suspension was observed for sedimentation, static and dynamic, pH, viscosity, and electrokinetic properties. The pH was measured with a line operated Beckman meter and a Hoeppler viscosimeter was used to measure the viscosity. The temperature of the suspension was held at 20° ± 0.2° during the viscosity measurement by means of a circulating water bath.

The two types of sedimentation studies were made in an attempt to determine the amount of settling and whether agglomeration was taking place. For the amount of settling, a 100-ml. graduated cylinder was filled to the mark with the suspension and sealed. These were then observed

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† Present address: Abbott Laboratories, North Chicago, Ill.

for settling. A dynamic sedimentation test was similar to the method described by Daniels, *et al.* (15). Ten milliliters of throughly redispersed suspension was diluted to 350 ml. with distilled water. An aluminum disk was suspended in the dilute suspension and a 5-mg. weight added to the balance. When the pointer returned to zero, the time was noted and another 5-mg. weight added. This was continued until a definite trend was established as the interest was only in the larger particles.

The electrokinetic properties were studied by measuring the zeta potential and critical potential. Zeta potential was calculated from the following formula (16):

$$Z = (4 \times \pi \times \eta \times \mu)/D$$

For the conductance measurement, a Kohlrausch type slide wire was used with an oscillograph to indicate the null point. The rate of movement of the particles was measured in a microelectrophoresis cell (17). Two hundred and twenty-five volts were imposed across the cell and the time of particle travel was measured with an electric timer measuring to the 0.1 second. Ten readings were taken, five in each direction, at the lower 21% level and averaged to obtain the particle rate of travel.

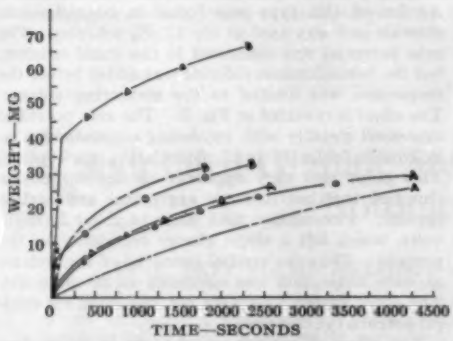

Fig. 1.—Sedimentation rate of 5% sulfamerazine. —△—methyl cellulose 25 c.p.s.; —○—methyl cellulose 4000 c.p.s.; ———CMC—medium viscosity.

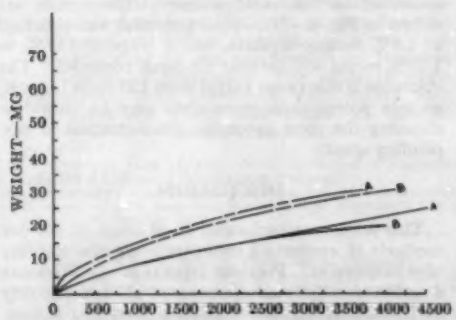

Fig. 2.—Sedimentation rate of 5% sulfamerazine. ———CMC—high viscosity, ———sodium alginate.

RESULTS

The sedimentation observations indicated that some marked changes had taken place. At the end of two months the methyl cellulose 4000 c. p. s. suspension had a cloudy supernatant layer with a caked sediment to the 7-ml. mark. This sediment could be redispersed with difficulty. A translucent zone above the 7-ml. mark was noted in the methyl cellulose 25 c. p. s. suspension. The sediment that rose to the 7-ml. mark could not be redispersed and had to be dug out of the graduate. Although the sodium alginate suspension settled to the 22-ml. mark, this sediment was easily and uniformly redispersed with no apparent caking. A sediment rising to the 10-ml. mark with a translucent supernatant layer was observed with the sodium carboxymethylcellulose medium viscosity suspension. However, this sediment was redispersed with slight shaking and no caking was apparent. No definite layer of powder could be noted with the carboxymethylcellulose high viscosity suspension, but there was a clear zone of supernatant liquid down to the 95-ml. mark. The suspension was easily resuspended with no apparent caking.

The rate of sedimentation test shows graphically what had taken place in the cylindrical graduates. Each suspension was thoroughly shaken to redisperse any sediment before a sample was removed. The methyl cellulose 25 c. p. s. suspension had caked severely and also had a large increase in the rate of sedimentation as shown in Fig. 1. Line *A* is the initial test and line *B* was determined four weeks later. Figure 1 also illustrates the caking that had taken place in the methyl cellulose 4000 c. p. s. suspension. A moderate amount of agglomeration had already taken place. The sedimentation rate of the sodium carboxymethylcellulose medium viscosity suspension also indicated the beginning of agglomeration (Fig. 1), while the high viscosity sodium carboxymethylcellulose suspension showed practically no change in sedimentation rate (Fig. 2). The sodium alginate suspension also remained uniformly dispersed as illustrated in Fig. 2.

These observations show that these suspensions greatly vary. The measurements of pH viscosity are shown in Tables I and II.

TABLE I.—pH OBSERVATIONS

	Initial	Two Weeks	Four Weeks
Methyl Cellulose, 25 c. p. s.	5.9	6.2	5.8
Methyl Cellulose, 4000 c. p. s.	5.3	5.5	5.6
Sodium Carboxymethyl-cellulose			
Medium viscosity	6.7	6.8	6.5
High viscosity	6.3	6.5	6.3
Sodium Alginate	5.9	6.0	5.7

With the exception of an unexplained decrease in viscosity of the high viscosity sodium carboxymethylcellulose suspension, the measurements of pH and viscosity did not indicate that any changes were taking place in the suspensions. This would seem to indicate that these factors do not predict the stability of a suspension.

TABLE II.—VISCOSITY OBSERVATIONS, C. P. S.

	Initial	One Week	Two Weeks	Four Weeks	Seven Weeks
Methyl Cellulose, 25 c. p. s.	7	7	7	7	7
Methyl Cellulose, 4000 c. p. s.	163	159	165	161	168
Sodium Carboxymethylcellulose					
Medium viscosity	38	39	37	37	36
High viscosity	264	222	222	181	174
Sodium Alginate	40	40	40	39	39

TABLE III.—ZETA POTENTIAL MEASUREMENTS

	Zeta Potential, mv.					
Suspending Agent	Initial	One Week	Two Weeks	Three Weeks	Five Weeks	Seven Weeks
Methyl Cellulose, 25 c. p. s.	39	9	20	19	21	24
Methyl Cellulose, 4000 c. p. s.	7	16	8	5	7	5
Sodium Carboxymethylcellulose						
Medium viscosity	133	174	157	104	124	113
High viscosity	80	142	116	201	139	142
Sodium Alginate	119	141	123	142	155	126

The electrokinetic factors were studied by measuring the zeta potential and critical potential. Each measurement was from an aged, undisturbed portion of the original suspension. Table III presents the zeta potentials observed.

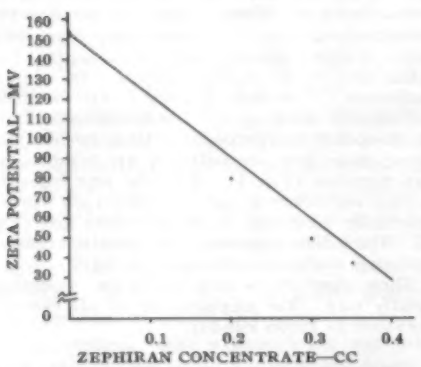

Fig. 3.—Critical potential of 5% sulfamerazine in sodium alginate 1%.

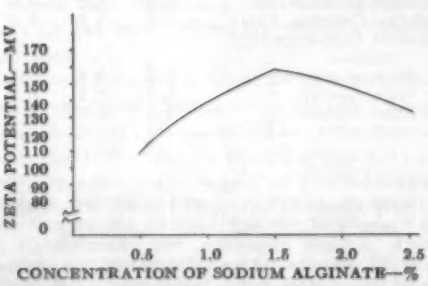

Fig. 4.—Zeta potential of 5% sulfamerazine in various concentrations of sodium alginate.

Critical potential is considered that zeta potential at which flocculation of the suspension occurs. It is not necessary to reduce the zeta potential to zero to obtain flocculation. As all of the suspensions in this study had negative charges, the addition of a highly positive ion could flocculate the suspension. An ion of this type was found in benzalkonium chloride and was used as the 12.8% solution. The zeta potential was measured in the usual manner, but the benzalkonium chloride was added before the suspension was diluted to the measuring volume. The effect is revealed in Fig. 3. The zeta potential decreased steadily with increasing amounts of benzalkonium chloride until flocculation took place. This point was very apparent as the suspension clumped together in large aggregates and settled rapidly. Flocculation took place at about 29 millivolts, which left a slight charge remaining on the particle. Thus the critical potential of the sodium alginate suspension was cosidered as 29 millivolts. The other suspensions were not measured for critical potential at this time.

The effect of various percentages of suspending agent on the zeta potential was measured by preparing a series of 5%-sulfamerazine suspensions with 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% sodium alginate. The viscosities and zeta potentials were measured in the usual manner. The results are shown in Fig. 4. The peak potential was obtained at 1.5% sodium alginate, but a range of 1.25% to 1.75% would still be near the peak potential. The viscosity in this range varied from 120 to 340 c. p. s., so zeta potential measurements may be an aid in choosing the most favorable concentration of suspending agent.

DISCUSSION

This work was performed to attempt to develop methods of evaluating and predicting the stability of a suspension. Previous reports in the literature mention that the measurements of pH and viscosity do not always indicate whether a particular suspension will or will not be stable. The measurements of pH and viscosity with these suspensions settled and caked without any change in these factors.

Electrokinetic factors, such as zeta potential and critical potential, seem to offer a method of evaluating and predicting the stability of a suspension. When the sulfamerazine suspensions were initially measured, the methyl cellulose preparations were much lower in zeta potential than the other suspensions. These suspensions settled and caked. The sodium carboxymethylcellulose medium viscosity suspension showed a decline in zeta potential and also an increase in the rate of sedimentation. By being able to discard immediately potentially unstable suspensions, as the methyl cellulose group, and being able to compare the suspensions during aging, as the sodium carboxymethylcellulose suspensions, the measurement of zeta potential appears to be of value in predicting and evaluating a series of suspensions.

It was shown that a stable suspension can be flocculated when the zeta potential is lowered below a critical level. This factor would be of value after the basic formula is chosen and other ingredients such as preservatives, dyes, and flavoring agents are to be added. These substances can adversely affect the stability and a consideration of the critical potential would offer a method of determining the most compatible combination to use. Also, the concentration of the suspending agent could be chosen by electrokinetic measurements.

The study of these factors proposes a method of screening and evaluating a series of suspensions so that the most stable preparation can be found. The other factors of pH and viscosity could be adjusted in relation to the electrokinetic factors. Therefore, if each suspension is an individual problem, sufficient measurements are needed to evaluate and compare various formulas.

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A Simplified Graphic Method for the Preparation of Isotonic Solutions*

By E. ROY HAMMARLUND† and KAJ PEDERSEN-BJERGAARD‡

A new simplified graphic method for the adjustment of isotonic solutions is presented. The use of this method replaces many of the arithmetical calculations which are usually necessary with other methods of adjustment. The amount of NaCl that is required to be added to each solution can be quickly and accurately determined for all concentrations. A revised table of NaCl equivalents for 332 different medicinal substances which are usually used in the preparation of isotonic solutions is presented. The table is more complete than previously used tables and it gives the NaCl equivalents at various concentrations.

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† Associate Professor of Pharmacy, School of Pharmacy, State College of Washington, Pullman.
‡ Apoteker, Valby Apotek, Copenhagen, Denmark.
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THE USE OF ISOTONIC SOLUTIONS has increased markedly in the last few years. Many methods have been proposed for calculating the amount of NaCl, or other substance, that must be added to a hypotonic solution to make it isotonic with blood and tears. Most of these methods make use of some type of NaCl equivalent which is a comparison of the osmotic effect of a solution of a medicinal substance to that of NaCl.

There are numerous tables of NaCl equivalents in the various pharmaceutical texts; but unfortunately, there are some discrepancies in these tables; depending upon the method of calculation for each substance, the freezing point used, and upon whether the values were theoretical or experimentally determined. Since these discrepancies exist in the presently used tables of NaCl equivalents, the purpose of this study is to investigate some of the colligative properties of all drugs whose aqueous solutions are used in collyria, collunaria, injections, or in other contact

with tissues; and to present the data both as a complete table of NaCl equivalents at various concentrations, and as a new, simplified graphic method of isotonicity adjustment. The substances tested were of U. S. P. or N. F. grade of purity or better, such as reagent grade, chemically pure, or at whatever grade of purity each specialty is marketed.

EXPERIMENTAL METHODS AND RESULTS

Pedersen-Bjergaard and co-workers (1–3) have previously reported the freezing point depressions of solutions of many medicinal compounds which were calculated from vapor pressure measurements using Baldes' modification (4) of Hill's thermoelectric method (5), or which were determined directly by means of a Beckman thermometer. The freezing point measurements were corrected for the amount of disengaged ice and −0.52° was found to be the freezing point of blood, tears, and of 0.9% NaCl solution (6, 7). The freezing point depression for various concentrations of 332 drugs has been determined using the method corrected for disengaged ice. Since all freezing point measurements of the drugs under study were made in comparison to those of a 0.9% NaCl solution, the data that are presented give solutions that are isotonic with normal saline solution and hence, also isotonic with blood and tears.

The data were initially plotted as freezing point depression *vs.* concentration of drug and the graph included a mirrored NaCl curve (1). A series of concentrations of each substance was selected and from the mirrored NaCl curve was obtained the amount of NaCl that is required to make each of these concentrations isotonic. The amount of NaCl required was plotted against the concentration of each substance in question and a new simplified graph of the type suggested by Baggesgaard-Rasmussen and Jerslev (8) was drawn for each of the medicinal substances. In this method the concentration of the substances to be made isotonic (in Gm./100 cc. or gr./fl. oz.) is plotted as the abscissa of a graph and the grams of NaCl required per 100 cc. of solution or grains NaCl per fluid ounce is plotted as the ordinate. The use of the graphs makes it possible for one to determine rapidly the approximate amount of NaCl for adjustment to isotonicity for all possible concentrations even though a NaCl equivalent may not be available for that particular concentration of drug. The fact that some of the graphs are curved lines shows that the freezing point depression is not always directly proportional to the amount of solute. Therefore, it is sometimes undesirable to use only a single NaCl equivalent for all concentrations as has been presented in previous tables, and a graph is a convenient method for obtaining the actual NaCl equivalency of a substance at all concentrations. The use of this graphic method has been shown to give sufficiently accurate results (9). The graphic methods of use are as follows:

Solutions of a Single Compound.—The concentration of the medicinal compound, expressed in percentage (Gm./100 cc. solution), is sought along the abscissa of the graph.[1] One moves vertically up the line of the proper concentration until it intersects the medicament curve and then moves either right or left to the ordinate and obtains the value of NaCl to be added in Gm./100 cc. or grains/fluid ounce of total solution. For the adjustment

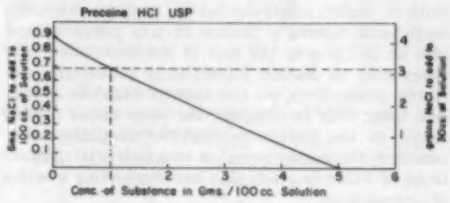

Figure 1

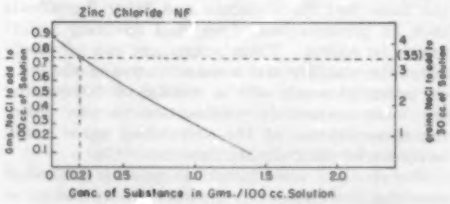

Figure 2

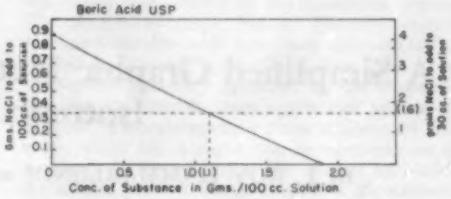

Figure 3

of solutions containing silver ions which are incompatible with NaCl, the graph designates the amount of sodium nitrate to add. Likewise, sodium sulfate should be used for the adjustment of butacaine sulfate solutions.

Example 1

℞ Procaine HCl 0.3 Gm.
Distilled H_2O *q. s. ad* 30.0 cc.
M. Ft. Isotonic Solution

Start from the point on the abscissa corresponding to the concentration of the medicament, 1%; now travel vertically until this concentration cuts the medicament curve; then travel horizontally either left or right depending upon which system of weights is being used. The intersection at the left border is at 0.69 Gm. NaCl/100 cc. of solution. Then,

[1] A booklet which contains the necessary individual graphs to be used for the simplified graphic method of isotonicity adjustment of solutions is being distributed by the authors, free of charge.

0.3 × 0.69 Gm. = 0.21 Gm. NaCl for the 30 cc. The intersection at the right border is at 3.2 grains NaCl/fluid ounce which is required to make the prescription isotonic.

This amount of NaCl in Gm. (or grains) which was found to be required can be converted easily to the equivalent number of cc. (or minims) of normal saline solution or any isotonic buffer solution by multiplying the Gm. (or grains) of NaCl needed by 111.1 (or 117.6) and obtaining the volume in cc. (or minims) of isotonic solution to add and then *q. s.* to the final volume with distilled water. This procedure enables one to use previously prepared buffered diluting solutions along with the simplified graphical method and requires only one simple arithmetical operation with a pencil and paper. For example, in the above prescription of Procaine HCl: 0.21 Gm. NaCl is required to make the 30 cc. isotonic and one wishes to use normal saline solution instead of granular NaCl for adjustment; 0.21 × 111.1 = 23.3 cc. normal saline solution (or any isotonic solution) to add; then *q. s.* from this volume up to 30 cc. with distilled water and the resulting solution will be isotonic.

Solutions Containing More Than One Compound.—If a solution contains two or more (n) medicinal compounds, the amounts of NaCl to be added in order to make each individual compound in the prescribed concentration isotonic are determined in the same manner as described before. In the metric system the sum (S) of the required amounts of NaCl (as determined from the individual graphs of (n) numbers of ingredients)—minus one less than the total number of ingredients ($n - 1$)—times 0.9 gives the Gm. NaCl which render the prescribed 100 cc. of solution approximately isotonic with blood and tears, i. e., $S - [(n - 1) \times 0.9]$ = Gm. NaCl required to make 100 cc. solution of (n) ingredients isotonic with blood and tears. This amount then may be converted to the corresponding amount of NaCl for the required volume of the prescription if it is different from 100 cc. In the apothecary system, the sum (S) of the determined amounts of NaCl in grains/fluid ounce minus $[(n - 1) \times 4.1]$ gives the grains of NaCl which renders one fluid ounce of the solution isotonic. If the result of the subtraction is a negative figure, however, it means the solution is already hypertonic and that no more NaCl should be added.

Example 2

℞ Zinc Chloride	gr. i
Boric Acid	gr. v
Distd. Water, *q. s. ad*	fl. ℥ i
M. Ft. Isotonic with NaCl	

Zinc Chloride, 1 gr/℥, is a 0.2% solution. Boric Acid, 5 gr./℥, is a 1.1% solution. 0.2% Zinc Chloride solution requires 3.5 grains NaCl/℥, or 0.76 Gm./100 cc. 1.1% Boric Acid solution requires 1.6 grains NaCl/℥, or 0.35 Gm./100 cc. Therefore,

3.5 gr/℥		0.76 Gm./100 cc.
1.6 gr/℥		0.35 Gm./100 cc.
5.1 gr. NaCl	or,	1.11 Gm. NaCl

is the sum of the two ingredients. Substitute in the formula: 5.1 gr. $-[(2 - 1) \times 4.1$ gr.$] = 5.1 - 4.1 = 1.0$ gr. NaCl required to be added/fl. ℥, or, 1.11 Gm. $-[(2 - 1) \times 0.9$ Gm.$] = 1.11 - 0.9 =$ 0.21 Gm. NaCl required/100 cc. solution. For 30 cc., it is 0.3 × 0.21 Gm. = 0.06 Gm. NaCl/30 cc.

The required amount of NaCl likewise can be converted to volume of any isotonic diluting solution by multiplying the weight of NaCl in Gm. (or grains) by 111.1 (or 117.6) and obtaining the cc. (or minims) of isotonic solution to add and then diluting to the final volume with distilled water.

1.0 gr./fluid ounce × 117.6 = 118 minims normal saline solution to add and then *q. s.* to ℥i with water, or, 0.06 Gm./30 cc. × 111.1 = 6.7 cc. normal saline solution to add and then *q. s.* to 30 cc. with water.

Table of Sodium Chloride Equivalents.—A revised table of NaCl equivalents at 1%, 3%, 5% concentrations, and at the concentration of isotonicity has been prepared on the basis of experimental freezing point determinations and is presented in Tables I and II. Table I gives the NaCl equivalents for those medicinal substances which normally are available to the pharmacist in pure chemical form for use in compounding. Table II gives the NaCl equivalents for those medicinal substances which normally are not available in pure chemical form but occur as prepared solutions and other specialties. One should use the NaCl equivalent which represents the concentration nearest to the desired concentration of medicinal substance used.

TABLE I.—SODIUM CHLORIDE EQUIVALENTS OF DRUGS USUALLY AVAILABLE IN PURE FORM.

Chemical	Concentration of solution — NaCl Equivalents — 1%	3%	5%	At Isotonicity
Acriflavine N. F.	0.10	0.09	..	..
Alcohol U. S. P.	0.65	..	..	0.65 (1.39%)
Alcohol, Dehydrated N. F.	0.70	..	..	0.70 (1.28%)
Alum (Potassium) N. F.	0.18	0.15	0.15	0.14 (6.35%)
Aminophylline U. S. P.	0.17	..	..	..
Amiodoxyl Benzoate	0.20	0.20	..	0.20 (4.42%)
Ammonium Chloride U. S. P.	1.12	..	..	1.12 (0.8%)
Amobarbital Sodium U. S. P.	0.25	0.25	..	0.25 (3.6%)
Amphetamine Phosphate N. F.	0.34	0.27	..	0.26 (3.47%)
Amphetamine Sulfate U. S. P.	0.22	0.21	..	0.21 (4.23%)
Amprotropine Phosphate	0.18	0.16	0.15	..
Amylcaine HCl	0.22	0.19	..	0.18 (4.98%)
Antimony Potassium Tartrate U. S. P.	0.18	0.13	0.10	..
Antipyrine N. F.	0.17	0.14	0.14	0.13 (6.81%)

TABLE I—(*Continued*)

Chemical	Concentration of solution — NaCl Equivalents 1%	3%	5%	At Isotonicity
Apomorphine HCl U. S. P.	0.14	..	..	..
Arecoline HBr N. F.	0.27	0.24	..	0.23 (3.88%)
Arsenic Trioxide N. F.	0.30	..	..	..
Ascorbic Acid U. S. P.	0.18	0.18	0.18	0.18 (5.04%)
Atropine Sulfate U. S. P.	0.13	0.11	0.11	0.10 (8.85%)
Aurothioglucose N. F.	0.03	0.03	0.03	..
Bacitracin U. S. P.	0.05	0.04	0.04	..
Barbital Sodium N. F.	0.30	0.29	..	0.29 (3.12%)
Benzyl Alcohol N. F.	0.17	0.15	..	..
Bismuth Potassium Tartrate N. F.	0.09	0.06	0.05	..
Bismuth Sodium Tartrate	0.13	0.12	0.11	0.10 (8.91%)
Boric Acid U. S. P.	0.50	..	..	0.47 (1.9%)
Butacaine Sulfate N. F.	0.20	0.13	0.10	..
Butethamine Formate	0.26	0.21	..	0.20 (4.56%)
Caffeine U. S. P.	0.08	..	..	..
Caffeine and Sodium Benzoate U. S. P.	0.26	0.23	..	0.23 (3.92%)
Caffeine and Sodium Salicylate N. F.	0.21	0.17	0.16	0.16 (5.77%)
Calcium Aminosalicylate U. S. P.	0.27	0.21	..	..
Calcium Chloride U. S. P.	0.51	..	..	0.53 (1.70%)
Calcium Chloride ($6H_2O$)	0.35	..	..	0.36 (2.5%)
Calcium Chloride, Anhydrous	0.68	..	..	0.69 (1.3%)
Calcium Gluconate U. S. P.	0.16	0.14	..	..
Calcium Lactate N. F.	0.23	0.21	..	0.20 (4.5%)
Calcium Levulinate N. F.	0.27	0.25	..	..
Calcium Pantothenate U. S. P.	0.18	0.17	0.17	0.16 (5.5%)
Chiniofon U. S. P.	0.13	0.11	..	..
Chloramine-T N. F.	0.23	0.22	..	0.22 (4.1%)
Chlorobutanol (Hydrated) U. S. P.	0.24	..	..	..
Chlortetracycline Sulfate	0.13	0.10	..	..
Citric Acid U. S. P.	0.18	0.17	0.16	0.16 (5.52%)
Cocaine HCl U. S. P.	0.16	0.15	0.14	0.14 (6.33%)
Codeine HCl	0.15	0.15	..	..
Codeine Phosphate U. S. P.	0.14	0.13	0.13	0.12 (7.29%)
Cupric Sulfate N. F.	0.18	0.15	0.14	0.13 (6.85%)
Cupric Sulfate, Anhydrous	0.27	0.23	..	0.22 (4.09%)
Dextroamphetamine Phosphate	0.25	0.25	..	0.25 (3.62%)
Dextrose U. S. P.	0.16	0.16	0.16	0.16 (5.51%)
Dextrose, Anhydrous	0.18	0.18	..	0.18 (5.05%)
Dibucaine HCl U. S. P.	0.13	0.11	0.08	..
Dihydrostreptomycin Sulfate U. S. P.	0.06	0.05	0.05	0.05 (19.4%)
Emetine HCl U. S. P.	0.10	0.10	0.10	..
Ephedrine HCl N. F.	0.30	0.28	..	0.28 (3.2%)
Ephedrine Sulfate U. S. P.	0.23	0.20	..	0.20 (4.54%)
Epinephrine Bitartrate U. S. P.	0.18	0.16	0.16	0.16 (5.7%)
Ergonovine Maleate U. S. P.	0.16	..	..	..
Ethaverine HCl	0.12	..	..	..
Ethylenediamine	0.44	..	..	..
Ethylhydrocupreine HCl	0.17	0.11	0.09	..
Ethylmorphine HCl U. S. P.	0.16	0.15	0.15	0.15 (6.18%)
Ferric Ammonium Citrate, Green N. F.	0.17	0.15	0.14	..
Ferrous Gluconate U. S. P.	0.15	0.12	0.11	..
Ferrous Lactate	0.21	..	..	..
Fluorescein Sodium U. S. P.	0.31	0.27	..	0.27 (3.34%)
D-Fructose	0.18	0.18	..	0.18 (5.05%)
Galactose	0.18	0.18	..	0.18 (4.92%)
D-Glucuronic Acid	0.20	0.19	..	..
L-Glutamic Acid	0.25	..	..	..
Glycerin U. S. P.	0.35	..	..	0.35 (2.6%)
Guanidine HCl	0.65	..	..	0.61 (1.47%)
Heparin Sodium U. S. P.	0.08	0.07	0.07	..
Hippuran®	0.16	0.15	0.15	0.15 (5.92%)
Holocaine® HCl	0.20	..	..	..
Homatropine HBr U. S. P.	0.17	0.16	0.16	0.16 (5.67%)
Homatropine Methylbromide U. S. P.	0.19	0.15	0.13	..
Hydrastine HCl	0.15	0.12	0.11	..
Hydroxyquinoline Sulfate	0.21	0.14	0.12	..
Hyoscyamine HBr N. F.	0.19	0.16	0.14	..
Hyoscyamine Sulfate N. F.	0.14	0.12	0.11	..
Intracaine HCl	0.23	0.20	..	0.18 (4.97%)
Isoniazid U. S. P.	0.25	0.22	..	0.21 (4.35%)

TABLE I—(*Continued*)

Chemical	Concentration of solution — NaCl Equivalents 1%	3%	5%	At Isotonicity
Lactic Acid U. S. P.	0.41	..	..	0.39 (2.3%)
Lactose U. S. P.	0.07	0.08	0.09	0.09 (9.75%)
Magnesium Chloride	0.45	..	..	0.45 (2.02%)
Magnesium Sulfate U. S. P.	0.17	0.15	0.15	0.14 (6.3%)
Mannitol N. F.	0.17	0.17	0.18	0.18 (5.07%)
Menadione Sodium Bisulfite U. S. P.	0.20	0.18	0.18	0.18 (5.07%)
Meperidine HCl U. S. P.	0.22	0.20	..	0.19 (4.80%)
Mephenesin N. F.	0.19	..	..	..
Merbromin N. F.	0.14	0.11	0.09	..
Mercuric Cyanide	0.15	0.14	0.13	..
Mercury Bichloride U. S. P.	0.13	0.12	0.10	..
Methacholine Chloride U. S. P.	0.32	..	..	0.28 (3.21%)
Methadone HCl U. S. P.	0.18	0.14	0.12	0.10 (8.59%)
Methamphetamine HCl U. S. P.	0.37	..	..	0.33 (2.75%)
Methenamine U. S. P.	0.23	0.24	..	0.25 (3.68%)
Methionine N. F.	0.28	..	..	..
Methylatropine Bromide	0.14	0.13	0.13	0.13 (7.03%)
Monoethanolamine N. F.	0.53	..	..	0.51 (1.76%)
Morphine HCl U. S. P.	0.15	0.14	..	..
Morphine Nitrate	0.19	0.15	..	..
Morphine Sulfate U. S. P.	0.14	0.11	0.09	..
Narcotine HCl	0.10	0.08	0.08	..
Neomycin Sulfate U. S. P.	0.11	0.09	0.06	..
Nicotinamide U. S. P.	0.26	0.21	..	0.20 (4.49%)
Nicotinic Acid U. S. P.	0.25	..	..	..
"Panthesine"	0.18	0.15	..	..
Papaverine HCl U. S. P.	0.10	..	..	..
Potassium Penicillin G U. S. P.	0.18	0.17	0.16	0.16 (5.48%)
Sodium Penicillin G U. S. P.	0.18	0.16	0.16	..
Pentobarbital Sodium U. S. P.	0.25	0.23	..	..
Pentylenetetrazole U. S. P.	0.22	0.19	..	0.18 (4.91%)
Phenindamine Tartrate U. S. P	0.17	0.12	0.10	..
Phenobarbital Sodium U. S. P.	0.24	0.23	..	0.23 (3.95%)
Phenol U. S. P.	0.35	..	..	0.32 (2.8%)
Phenylpropanolamine HCl	0.38	..	..	0.35 (2.6%)
Physostigmine Salicylate U. S. P.	0.16	..	..	..
Physostigmine Sulfate	0.13	0.12	0.12	0.12 (7.74%)
Pilocarpine HCl U. S. P.	0.24	0.22	..	0.22 (4.08%)
Pilocarpine Nitrate U. S. P.	0.23	0.20	..	..
Polymyxin B Sulfate U. S. P.	0.09	0.06	0.04	..
Potassium Chlorate N. F.	0.49	..	..	0.48 (1.88%)
Potassium Chloride U. S. P.	0.76	..	..	0.76 (1.19%)
Potassium Iodide U. S. P.	0.34	..	..	0.34 (2.59%)
Potassium Nitrate N. F.	0.56	..	..	0.56 (1.62%)
Potassium Permanganate U. S. P.	0.39	..	..	..
Potassium Phosphate N. F.	0.46	..	..	0.43 (2.08%)
Potassium Phosphate, Monobasic	0.44	..	..	0.41 (2.18%)
Potassium Sulfate	0.44	..	..	0.43 (2.11%)
Procainamide HCl U. S. P.	0.22	0.19	0.17	..
Procaine HCl U. S. P.	0.21	0.19	0.18	0.18 (5.05%)
Propylene Glycol U. S. P.	0.45	..	..	0.45 (2.0%)
Pyridoxine HCl U. S. P.	0.37	0.29	..	..
Quinidine Sulfate U. S. P.	0.10	..	..	..
Quinine Bisulfate N. F.	0.09	0.09	..	..
Quinine Dihydrochloride N. F.	0.23	0.19	0.18	0.18 (5.07%)
Quinine Hydrochloride U. S. P.	0.14	0.11	..	..
Quinine and Urea Hydrochloride N. F.	0.23	0.21	..	0.20 (4.5%)
Racephedrine HCl N. F.	0.31	0.30	..	0.29 (3.07%)
Resorcinol U. S. P.	0.28	0.27	..	0.27 (3.3%)
Scopolamine HBr U. S. P.	0.12	0.12	0.12	0.11 (7.85%)
Scopolamine Methylnitrate	0.16	0.14	0.13	0.13 (6.95%)
Secobarbital Sodium U. S. P.	0.24	0.23	..	0.23 (3.9%)
Silver Nitrate U. S. P.	0.33	..	..	0.33 (2.74%)
Mild Silver Protein N. F.	0.17	0.17	0.16	0.16 (5.51%)
Strong Silver Protein N.F.	0.08	0.05	0.04	..
Sodium Acetate, Anhydrous	0.77	..	..	0.76 (1.18%)
Sodium Acetate N. F.	0.46	..	..	..
Sodium Aminosalicylate U. S. P.	0.29	0.28	..	0.27 (3.27%)
Sodium Antimonyl Tartrate	0.13	0.12	0.12	0.11 (7.9%)
Sodium Arsenate, Dibasic	0.25	0.24	..	0.24 (3.83%)
Sodium Ascorbate	0.33	0.30	..	0.30 (3.0%)

TABLE I—(*Continued*)

Chemical	Concentration of solution — NaCl Equivalents — 1%	3%	5%	At Isotonicity
Sodium Benzoate U. S. P.	0.40	..	..	0.40 (2.25%)
Sodium Bicarbonate U. S. P.	0.65	..	..	0.65 (1.39%)
Sodium Biphosphate, Anhydrous	0.46	..	..	0.43 (2.10%)
Sodium Biphosphate, U.S.P.	0.40	..	..	0.37 (2.45%)
Sodium Biphosphate ($2H_2O$)	0.36	..	..	0.32 (2.77%)
Sodium Bisulfite U. S. P.	0.61	..	..	0.60 (1.5%)
Sodium Borate U. S. P.	0.42	..	..	0.35 (2.6%)
Sodium Bromide U. S. P.	0.57	..	..	..
Sodium Cacodylate N. F.	0.32	0.28	..	0.27 (3.3%)
Sodium Carbonate, Anhyd.	0.70	..	..	0.68 (1.32%)
Sodium Carbonate, Monohydrated U. S. P.	0.60	..	..	0.58 (1.56%)
Sodium Chloride U. S. P.	1.00	1.00	1.00	1.00 (0.9%)
Sodium Citrate U. S. P.	0.31	0.30	..	0.30 (3.02%)
Sodium Hypophosphite N. F.	0.61	..	..	..
Sodium Iodide U. S. P.	0.39	..	..	0.38 (2.37%)
Sodium Lactate	0.55	..	..	0.52 (1.72%)
Sodium Metabisulfite	0.67	..	..	0.65 (1.38%)
Sodium Nitrate	0.68	..	..	0.66 (1.36%)
Sodium Nitrite U. S. P.	0.84	..	..	0.83 (1.08%)
Sodium Phosphate Exsiccated, N. F.	0.53	..	..	0.51 (1.75%)
Sodium Phosphate N. F.	0.29	0.27	..	0.27 (3.33%)
Sodium Phosphate, Dibasic, ($2H_2O$)	0.42	..	..	0.40 (2.23%)
Sodium Phosphate, Dibasic, ($12H_2O$)	0.22	0.21	..	0.20 (4.45%)
Sodium Propionate N. F.	0.61	..	..	0.61 (1.47%)
Sodium Riboflavin Phosphate	0.08	0.08	..	..
Sodium Salicylate U. S. P.	0.36	..	..	0.36 (2.53%)
Sodium Sulfate N. F.	0.26	0.23	..	0.23 (3.95%)
Sodium Sulfate, Anhydrous	0.58	..	..	0.56 (1.61%)
Exsiccated Sodium Sulfite N. F.	0.65	..	..	..
Sodium Thiosulfate N. F.	0.31	..	..	0.30 (2.98%)
Sorbitol ($^1/_2 H_2O$)	0.16	0.16	0.16	0.16 (5.48%)
Stibamine Glucoside	0.14	0.11	..	..
Streptomycin Sulfate U. S. P.	0.07	0.06	0.06	..
Strychnine HCl	0.18	..	..	..
Strychnine Nitrate N. F.	0.12	..	..	..
Sucrose U. S. P.	0.08	0.09	0.09	0.10 (9.25%)
Sulfacetamide Sodium U. S. P.	0.23	0.23	..	0.23 (3.85%)
Sulfadiazine Sodium U. S. P.	0.24	0.22	..	0.21 (4.24%)
Sulfamerazine Sodium U. S. P.	0.23	0.21	..	0.20 (4.53%)
Sulfapyridine Sodium	0.23	0.21	..	0.20 (4.55%)
Sulfathiazole Sodium N. F.	0.22	0.20	..	0.19 (4.82%)
Tannic Acid N. F.	0.03	0.03	0.03	..
Tartaric Acid N. F.	0.25	0.23	..	0.23 (3.9%)
Tetracaine HCl U. S. P.	0.18	0.15	0.12	..
Tetraethylammonium Bromide	0.33	0.28	..	0.28 (3.17%)
Tetraethylammonium Chloride	0.34	..	..	0.33 (2.67%)
Theophylline U. S. P.	0.10	..	..	..
Thiamine HCl U. S. P.	0.25	0.22	..	0.21 (4.24%)
Tubocurarine Chloride U. S. P.	0.13	0.10	0.09	..
Urea U. S. P.	0.59	..	..	0.55 (1.63%)
Urethan U. S. P.	0.31	..	..	0.31 (2.93%)
Viomycin Sulfate	0.08	0.07	0.07	..
Zinc Chloride N. F.	0.61	..	..	..
Zinc Phenolsulfonate N. F.	0.18	0.17	0.17	..
Zinc Sulfanilate	0.21	0.19	0.18	..
Zinc Sulfate U. S. P.	0.15	0.13	0.12	0.12 (7.65%)
Zinc Sulfate, Dried	0.23	0.21	..	0.20 (4.52%)

TABLE II.—SODIUM CHLORIDE EQUIVALENTS OF DRUGS NOT USUALLY AVAILABLE IN PURE FORM

Chemical	Concentration of Solution — NaCl Equivalents — 1%	3%	5%	At Isotonicity
Adrenalone HCl	0.27	0.22	..	0.21 (4.24%)
9-Aminoacridine HCl	0.17	..	..	..
Amydricaine HCl	0.24	0.18	0.16	0.16 (5.74%)
Amydricaine Nitrate	0.19	0.17	0.16	0.16 (5.68%)
Antazoline HCl U. S. P.	0.23	..	..	..

TABLE II—(*Continued*)

Chemical	Concentration of Solution — NaCl Equivalents 1%	3%	5%	At Isotonicity
Antazoline Phosphate	0.20	0.17	0.15	..
Aranthol®	0.23	0.23	..	0.23 (3.96%)
Atropine Methyl Nitrate	0.18	0.15	0.14	0.14 (6.52%)
Benoxinate HCl	0.18	0.14	..	..
Benzalkonium Chloride U. S. P.	0.16	0.14	0.13	..
Benzethonium Chloride U. S. P.	0.05	0.02	0.02	..
Benzpyrinium Bromide	0.20	0.18	0.17	..
Bromodiphephydramine HCl	0.17	0.10	0.07	..
Butethamine HCl N. F.	0.25	..	..	..
Carbachol U. S. P.	0.36	..	..	0.32 (2.82%)
Cetyltrimethyl Ammonium Bromide	0.09	0.09	0.08	..
Chlorcyclizine HCl U. S. P.	0.17	0.09	0.07	..
Chloresium®	0.10	0.06	0.05	..
Chlorpheniramine Maleate U. S. P.	0.17	0.12	0.09	..
Chlorpromazine HCl	0.10	0.05	0.03	..
Cornecaine®	0.18	0.15	0.13	0.12 (7.30%)
Cyclomethycaine Sulfate	0.13	0.10	0.09	..
Cyclopentamine HCl	0.36	..	..	0.34 (2.68%)
Cyclopentolate HCl	0.20	0.18	0.17	..
Decamethonium Bromide	0.25	0.20	0.18	0.18 (5.0%)
Dibutoline Sulfate	0.16	0.15	0.14	..
Dichlorophenarsine HCl	0.55	..	..	0.55 (1.64%)
Dicyclomine HCl	0.18	0.17	..	..
Diethylcarbamazine Citrate	0.14	0.14	0.14	0.14 (6.29%)
Dihydrocodeinone Enolacetate HCl	0.14	0.13	0.12	0.12 (7.76%)
Dihydrohydroxycodeinone	0.14	0.13	0.13	0.12 (7.4%)
Dihydromorphinone HCl U. S. P.	0.22	0.17	0.15	0.14 (6.39%)
Diphenhydramine HCl U. S. P.	0.28	0.20	0.17	..
Diphenmethanil Methylsulfate	0.15	..	..	..
Dipyrone®	0.19	0.19	..	0.19 (4.65%)
Edrophonium Chloride	0.31	0.27	..	0.27 (3.36%)
Ephedrine Lactate	0.26	0.24	..	0.24 (3.72%)
Epinephrine HCl	0.29	0.26	..	0.26 (3.47%)
Erythromycin Glucoheptonate	0.07	0.07	0.07	..
Ethylnorepinephrine HCl	0.32	0.28	..	0.27 (3.32%)
Evans Blue U. S. P.	0.06	0.05	0.05	..
Ferric Cacodylate N. F.	0.09	..	..	..
Gallamine Triethiodide	0.08	0.08	0.08	..
Glucosulfone Sodium	0.16	0.13	0.13	..
Glyphylline	0.12	0.10	0.09	0.08 (10.87%)
Hexamethonium Bromide	0.22	0.19	..	0.18 (4.99%)
Hexamethonium Chloride	0.27	0.27	..	0.27 (3.3%)
Hexobarbital Sodium N. F.	0.26	0.24	..	0.23 (3.88%)
Hexylcaine HCl	0.26	0.22	..	..
Histalog®	0.51	..	..	0.47 (1.91%)
Histamine Phosphate U. S. P.	0.25	0.23	..	0.22 (4.1%)
Histidine Monohydrochloride N. F.	0.29	0.26	..	..
4-Homosulfanilamide HCl	0.28	0.25	..	..
Hydralazine HCl	0.37	..	..	..
Hydroxyamphetamine HBr U. S. P.	0.26	0.25	..	0.24 (3.71%)
Iodophthalein Sodium U. S. P.	0.17	0.12	0.11	0.09 (9.58%)
Iodopyracet U. S. P.	0.11	0.11	0.10	0.10 (9.21%)
Iodopyracet Diethylamine	0.12	0.11	0.11	0.10 (8.73%)
Lidocaine HCl N. F.	0.22	0.21	..	0.20 (4.42%)
Lobeline HCl	0.16	..	..	..
Menadione Diphosphate	0.25	0.22	..	..
Mephenteramine Sulfate U. S. P.	0.22	0.20	..	0.19 (4.74%)
Mercaptomerin Sodium U. S. P.	0.18	0.18	0.18	..
Mercurophylline U. S. P.	0.13	0.10	0.09	..
Mersalyl U. S. P.	0.12	0.11	0.10	0.10 (9.06%)
Methacholine Bromide	0.28	0.24	..	0.24 (3.77%)
Methantheline Bromide U. S. P.	0.15	0.09	0.07	..
Methapyrilene HCl U. S. P.	0.19	0.18	0.17	..
Methoxamine HCl U. S. P.	0.26	0.24	..	0.24 (3.82%)
Naphazoline HCl N. F.	0.27	0.24	..	0.22 (3.99%)
Naphuride Sodium®	0.10	0.10	0.10	..
Neoarsphenamine	0.40	..	..	0.39 (2.32%)
Neostigmine Bromide U. S. P.	0.22	0.19	..	..
Neostigmine Methylsulfate U. S. P.	0.20	0.18	0.17	0.17 (5.22%)
Nikethamide U. S. P.	0.18	0.16	0.15	0.15 (5.94%)
Novaldin®	0.25	0.20	0.18	0.18 (5.0%)

TABLE II—(*Continued*)

Chemical	Concentration of solution — NaCl Equivalents — 1%	3%	5%	At Isotonicity
Oxophenarsine HCl U. S. P.	0.24	0.24	..	0.24 (3.67%)
Oxytetracycline HCl U. S. P.	0.13	0.08	..	..
Pentolinium Tartrate	0.17	0.15	0.15	..
Phenarsone Sulfoxylate	0.33	0.29	..	0.29 (3.07%)
Pheniramine Maleate N. F.	0.16	0.14	0.13	..
Phenylephrine HCl U. S. P.	0.32	0.30	..	0.30 (3.0%)
Phenylephrine Tartrate	0.19	0.16	0.16	0.15 (5.9%)
Phenylpropylmethylamine	0.38	..	..	0.33 (2.7%)
Piperocaine HCl U. S. P.	0.21	0.19	0.17	..
Piridocaine HCl	0.24	..	..	..
Plaquenil® Phosphate	0.18	0.15	0.13	..
Pramoxine HCl	0.18	0.15	0.10	..
Probarbital Calcium	0.25	..	..	..
Probarbital Sodium N. F.	0.32	0.29	..	0.29 (3.1%)
Promethazine HCl N. F.	0.18	0.10	0.07	..
Propoxycaine HCl	0.19	0.16	0 15	..
Pyrilamine Maleate U. S. P.	0.18	0.11	0.09	..
Quinacrine HCl U. S. P.	0.18	..	..	..
Quinacrine Methanesulfonate	0.11	0.10	0.10	..
Quinidine Gluconate N. F.	0.12	0.10	..	..
Sodium Folate	0.12	0.10	..	..
Sodium Ricinoleate	0.10	0.09	0.09	..
Sodium Sulfadimidine	0.21	0.19	0.18	..
Stibophen U. S. P.	0.18	0.16	0.15	..
Streptomycin Calcium Chloride Complex	0.20	0.19	0.18	0.18 (5.0%)
Streptomycin HCl	0.17	0.16	0.16	..
Succinylcholine Chloride U. S. P.	0.20	0.20	..	0.20 (4.48%)
Sulfisoxazole Diethanolamine	0.18	0.15	..	..
Sympocaine® HCl	0.18	0.15	0.14	..
Synkamin® HCl	0.32	..	..	..
Synthenate® Tartrate	0.19	0.17	0.15	..
Tetracycline HCl U. S. P.	0.14	0.10	..	..
Tetrahydrozoline HCl	0.28	0.23	..	..
Thiopental Sodium U. S. P.	0.27	0.26	..	0.26 (3.5%)
Tolazoline HCl U. S. P.	0.34	0.30	..	0.30 (3.05%)
Transentine® HCl	0.22	0.15	0.12	..
Tribromoethanol U. S. P.	0.05	..	..	..
Trimethaphan Camphorsulfonate	0.10	0.09	0.09	..
Triplennamine HCl U. S. P.	0.30	0.20	..	..
Tropacocaine HCl	0.25	0.20	..	0.18 (4.92%)
Tryparsamide U. S. P.	0.20	0.20	..	0.19 (4.62%)
Tuaminoheptane Sulfate N. F.	0.27	0.27	..	0.26 (3.4%)
Unan®	0.12	..	..	..
Vinbarbital Sodium	0.26	0.25	..	0.25 (3.55%)

SUMMARY

A simplified graphic method for the adjustment of isotonic aqueous solutions has been devised.

A revised table of NaCl equivalents at various concentrations for 332 different medicinal substances which are used in the preparation of isotonic solutions is presented.

The percentage concentration (w/v) of the solute, which when used alone will produce an aqueous solution that is isotonic with blood and tears, is also given in the table.

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The Kinetics of Rectal Absorption I.*

Preliminary Investigations into the Absorption Rate Process

By SIDNEY RIEGELMAN and WILFRED J. CROWELL†

The details of a radiological procedure are presented by which it is possible to conduct continuous external detection of the rate of rectal absorption of radio-tagged compounds from the specially designed suppository vehicles inserted in the rectal passage of a female rat. An analysis of the rate data is presented and a mathematical expression is derived which corrects the activity detected by the counter for the effect of the diffusion of the drug within the vehicle and for the accumulation of the absorbed drug in the scanned volume segment of the animal. Experimental results on three radio iodide-131 tagged compounds are presented and appear to confirm the resulting pseudo-first order rate equation.

THE MEDICINAL USE of suppositories dates back to the time of Hippocrates in which a vehicle consisting of soap and honey was used for administering cathartic compounds. Active interest in this form of medication developed during the nineteenth century, and since that time, considerable research has appeared both on individual bases and the drugs used therein. From studies based on individual drugs, clinicians have concluded that suppositories usually result in a low rate of drug release and in an erratic rate of absorption when compared to oral or other means of drug administration. Academic researchers, in contrast, have maintained that the rectal route possesses certain theoretical physiological advantages which should enhance the rate of drug absorption over rate obtained by the oral route and that it should be utilized to a much greater extent in modern therapeutics (1–3).

Considerable attention has been given in the literature to research which has endeavored to modify the properties and physical characteristics of cocoa butter, glycerinated gelatin, polyoxyethylene polymers, and numerous other bases. A review of these studies has been published recently by Gross and Becker (4).

Both *in vivo* and *in vitro* procedures have been used to characterize the advantages or disadvantages of proposed formulations and to compare the rectal route to other modes of drug administration. *In vitro* studies have been used to evaluate the rate of drug release by microbiological agar cup methods (5, 6) or by periodically determining the amount of drug dissolving in an aqueous medium from an immersed suppository (7, 8). Such studies confirm the postulate that water miscible formulations release medicaments at a faster rate than oleaginous bases. However, the rate differences obtained in these studies are of little value in predicting rates of rectal absorption since the solution rate process *in vivo* markedly differs from that in *in vitro* studies.

In vivo studies may be divided into two groups: one group comprising what may be called single point absorption studies; the other group, multipoint absorption or true rate studies. In general, single point studies are based on certain pharmacological end points, such as: loss of the righting reflex due to sedation (9), onset of curarization (10), or a single blood (11) or urine (12, 13) drug level. In contrast to single point absorption determinations, a number of multi-point absorption studies have been conducted to evaluate at different time intervals the level of a drug in the blood (14–19), urine (20), or in selected tissues (15, 16, 18); or to measure peripheral rise in temperature following the rectal administration of methyl nicotinate (21, 22).

Although these single point and multi-point *in vivo* absorption studies present much meaningful data, they provide an indirect approach to the absorption problem. They are based on the assumption that the rate of rectal absorption bears a constant, direct relationship to the amount of drug concentrated by some organ, or the blood level required to elicit a pharmacological response. Such studies provide information suitable only for gross comparisons; not one of them includes a direct procedure for continuous detection of the rate of absorption of the drug occurring at its site of insertion.

This study presents details of a method which was developed to provide direct and continuous detection of the rate of absorption of compounds tagged with a radioactive gamma emitter, I-131, from the rectum of an intact rat. The method

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This paper is based in part on a thesis submitted to the Graduate School of the University of California by W. J. Crowell in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
† Parke, Davis Fellow. Present Address: University of Southern California School of Pharmacy, Los Angeles.

results in a high degree of reproducibility of results. Analysis of the rate process is presented and the evidence appears to confirm a pseudo-first order system.

EXPERIMENTAL

Reagents.—Iodoform, Mallinckrodt, recrystallized twice from 95% ethanol, m. p. 118–120°; 2,4,6-triiodophenol, Eastman, recrystallized from 95% ethanol, m. p. 155.5–158°; Tween 20, Atlas Powder Co., and Antarox® A400,[1] General Dyestuffs Corp., were individually heated to 60° under reduced pressure (0.3 mm. Hg) to remove moisture and any low boiling fractions. Each compound was characterized by estimating the molecular weight from a measurement of the freezing point of a benzene solution of the surfactant. Polyethylene glycol 6000 pharmaceutical grade, Carbon & Carbide Co., was crystallized once from a solvent system containing 20% anhydrous ether and 80% chloroform. The degree of purity of both the crystallized and uncrystallized samples was determined by freezing point lowering measurements made with a calibrated thermistor-resistance meter (23). The molecular weight of the polyethylene glycol calculated from these values indicated a compound with a molecular weight considerably less than 6000. Polyethylene glycol 600, Carbon and Carbide Co., pharmaceutical grade; methylcellulose 6000 c.p.s. grade, Dow Chemical Co.; sodium carboxymethylcellulose high viscosity grade, Hercules Powder Co.

Radiological Equipment and Procedures.—All of the work done with radioactive isotopes was confined to the Radioactivity Center of the University of California Medical Center. Detection of the gamma radiation of sodium iodide-131 was accomplished by use of a scintillation counter composed of a shielded RCA 5819 photomultiplier which sensed the scintillations excited by the radiations illicited by the radio-tagged dose in a thallium activated sodium iodide crystal. This signal was coupled to a preamplifier and fed through a Tracer Laboratory Autoscaler, model SC–1B (Fig. 1). Due to the complex gamma decay scheme of iodide-131, it is not possible to give an absolute efficiency rating to the equipment, but it was in the order of magnitude of 20%.

The actual labeling of iodoform (IF) and triodophenol (TIP) with iodine-131 was carried out in a well ventilated fume hood, the sides and front of which were lined with lead bricks to a height of 22 inches. The lead floor of the hood was covered with stainless steel trays lined with strips of parafilm so that an accidental spill might be conveniently confined and the working area readily decontaminated. Centrifugation procedures were conducted in the reaction area and the supernatant liquids and washings collected in wide-mouth, glass-stoppered containers which were stored in a lead vault for disposal purposes. Surgical gloves were worn and the manipulations were carried out using a special pair of tongs (a test tube holder was clamped to the jaws of a pair of surgical tongs to facilitate the handling of small containers of radioactive material).

Fig. 1.—A photograph illustrating the position of the anesthetized rat, placed over the collimator-scintillation counter so as to obtain maximal, continuous external detection of a gamma-emitting dose. The scintillation counter, enclosed by lead brick shielding to minimize background counting rate, is electrically connected to the autoscaler and to the timer at the left of the rat.

The scale of reaction for the preparation of IF and TIP was calculated by dividing the total activity of I-131 by the specific activity required for the rectal absorption studies.

Scale of reaction—total activity available/required specific activity—(4 × 1000 mcc.)/200 mcc./mg.—20 mg.

Synthesis of Iodoform (IF).—Labeled IF was prepared according to the method of Vogel (24) by transferring a calculated volume of I-131 of total activity of 3 to 4 mc. to a 12-ml. centrifuge tube. A 0.25-ml. quantity of an aqueous preparation containing 25 mg. of sodium iodide (1.67×10^{-4} *M*) and 3.95 mg. of acetone (6.8×10^{-4} *M*) was added to the radioactive solution, followed by the addition of 0.40 ml. of 5% sodium hypochlorite solution.

After one hour the precipitated IF was collected by centrifugation and washed successively with a small volume of water and a 5% solution of potassium iodide. The IF was then dissolved in approximately 0.2 ml. of hot 50% aqueous ethanol. This solution was cooled in an ice bath to effect crystallization and the IF again collected by centrifugation, m. p. 118–120°; radioactive iodide impurities, 1–2%. The compound was dissolved in polyethylene glycol 600 for safe storage.

Synthesis of Triiodophenol (TIP).—The synthesis of labeled TIP was carried out according to a modification of the method of Marsh (25) by transferring a calculated volume of NaI-131 of total activity of 3 to 4 mc. to a 12-ml. centrifuge tube containing 2 ml. of glacial actic acid, 19 mg. sodium iodide (12×10^{-4} *M*) and 4.0 mg. of phenol (4.2×10^{-4} *M*). To the clear solution, 0.05 ml. of 30% hydrogen peroxide was added with a minimum of stirring. After a twenty-four hour interval, 8 ml. of water was added to the reaction mixture. The precipitated TIP was collected by centrifugation and washed successively with 2-ml. volumes of 5% potassium iodide aqueous solution.

Following the washing and centrifuging procedures, the TIP was dissolved in approximately 1

[1] This compound is a polyethylene glycol ether of nonyl phenol and is now sold under the trade name of Igepel® CO 630.

ml. of hot aqueous ethanol solution, allowed to crystallize in an ice bath, and was collected by centrifugation; m. p. 155.5–158°; radioactive iodide impurities, a mere trace. The compound was dissolved in polyethylene glycol 600 for safe storage.

Method for Determining Radiochemical Purity of Labeled Compounds.—The radiochemical purity of IF and TIP was determined by ascending paper partition chromatography, followed by analysis of the chromatogram using radioautographic techniques. Pure benzene was used as the solvent. Approximately 5 microcuries of labeled substance, dissolved in equal parts of ethanol and PEG 600, was applied to the base of the Whatman No. 1 filter paper and chromatographed for three hours. The solvent benzene at the base of the sealed jar was monitored for activity and found to possess negligible amounts. The paper strip was carefully dried and placed in contact with an x-ray film for one hour. The darkened areas on the developed film were used as a criterion for cutting the corresponding chromatogram into two nearly equal parts, one part containing the base line and the other part containing the labeled compound. The individual parts were folded several times, stapled to assume a fixed geometry, and monitored by means of a scintillation counter. Impurities in the sample, such as radioactive iodide, were then determined by expressing counts per second for the baseline portion as a per cent of the pooled count recorded for the entire paper strip.

Method for Determining the Activity of Labeled Compounds.—Since both IF and TIP are slightly volatile compounds, no attempt was made to dry them to a constant weight nor to standardize them by doing an absolute beta count. The activities, however, were determined relative to a solution of NaI–131, standardized by absolute beta count, which was transferred in volumetric increments to several 12-ml. centrifuge tubes containing milligram quantities of inert sodium iodide. The increments, covering a range of 8 to 500 microcuries of I–131, were assayed for gamma radiation by placing them individually in a lead container on the collimator of a standardized (RaD-RaE) scintillation counter. By plotting counts per second *versus* known activity on log-log coordinate paper, a graph was obtained for the conversion of IF and TIP gamma emissions into I-131 microcuries equivalents.

Pharmacological Procedures.—Female Sprague-Dawley rats, weighing 180 to 205 Gm., were anesthetized with sodium pentobarbital in a 20 mg. per ml. solution by interperitoneal injection in concentrations of 4.3 to 4.7 mg. per 100 Gm. body weight. In the majority of cases surgical anesthesia was complete in seven to fifteen minutes and persisted for about ninety minutes. At that time the experimental procedure was terminated and the animals were sacrificed by administering a lethal dose of undiluted pentobarbital solution.

Prior to the experiment the fecal content in the rectal canal was reduced by fasting the rat for thirty-six to forty-eight hours.[2] At the end of the fasting period, the rat was anesthetized and its rectum prepared by inserting a moistened plug to a depth of 2.5 cm. using a wetted solid-glass rod applicator. This plug, referred to as the proximal rectal plug, was constructed by inserting a glass bead (3.5 mm. in diameter) into a small portion of rubber tubing. The viscous suppository vehicles were retained in the rectum by the distal plug which was constructed by pulling a trimmed rubber policeman over a hollow glass tube (2 mm. in diameter). This plug was inserted into the anal canal to a depth of one centimeter and held in place by adhesive tape. The combination of plugs left a cavity of 1.5 cm. into which the dose of 0.2 ml. was inserted.

Fig. 2.—A photograph showing the proximal and distal rectal plugs used to confine a radiotagged dose to a definite length of the rat's rectal canal. The scanning field of the counter is delected by the crossed sticks placed in the one-inch cylindrical lead collimator.

After preparing the rectum, the anesthetized rat was mounted in the supine position with its legs stretched and taped to a plexiglass board (12 in. × 5 in. × 0.2 in.). The board and rat were then placed over a one-inch collimator in the desired geometrical position as determined in preliminary studies to result in maximal counting from the rectal area (Fig. 2). A 0.2 ml. dose was drawn up into a 1-ml. tuberculin syringe fitted with a No. 17 gauge needle. The needle was slipped into the glass tube portion of the distal plug, and forced past the seal of the policeman. In order to minimize the possibility of perforating the wall of the colon, the length of the needle was modified. After injecting the dose, the needle was withdrawn.

Counting Procedure.—A few seconds after introducing the radioactive preparation into the rectal canal, the autoscaler was activated and the counting recorded at one minute intervals for the first twenty minutes, and at five minute intervals for the remainder of the experiment. The scaler was automatically activated by a cam arrangement at one- and five-minute intervals by means of an electronic device driven by a small synchronous motor. Counts per second *versus* time were then plotted on semilog paper for the first ten minutes and extrapolated to zero time. The extrapolated value obtained was taken as 100% of the total dose administered and was used to express all other counts on a per cent basis.

Formulas for Suppository Vehicles.—In the sodium iodide studies, 5 microcuries of NaI-131 was added for each 0.2 ml. of vehicle used. The basic

[2] The animals were given only water during this period.

formula for the methylcellulose (MC) vehicle and the carboxymethylcellulose (CMC) vehicle is as follows:

Methylcellulose, 4000 c. p. s. or Carboxymethylcellulose high viscosity type	1.5 Gm.
Sodium iodide (carrier)	0.167 Gm.
Sodium bisulfite	0.050 Gm.
Sodium chloride	*q.s.* to make isotonic
Phosphate buffer (0.05 *M*, pH 7.4)	*q.s.* to make 100.0 cc.

Special MC and CMC vehicles were formulated to contain specified amounts of the nonionic surfactants, Tween 20 (TW 20) and Antarox A400 (A400). These were added to the above vehicles in the required amounts and corrected for, in the tonicity adjustment, and in the quantity of gum added to bring the preparation to constant viscosity. The CMC vehicle was used with the A400 system since a coacervate formed when this surfactant was used in the MC vehicle.

The iodoform was incorporated in a special isotonic liquid polyethylene glycol vehicle (LPEG) at a level of radioactivity of 5 microcuries per 0.2 ml. of vehicle used; polyethylene glycol 6000—59.0 Gm.; polyethylene glycol 600—5.0 Gm.; distilled water—*q.s.* to make 100.0 cc.

Triiodophenol was added to a buffered methylcellulose vehicle H in such a way that the sodium phenolate ion was formed (NaTIP). Once again

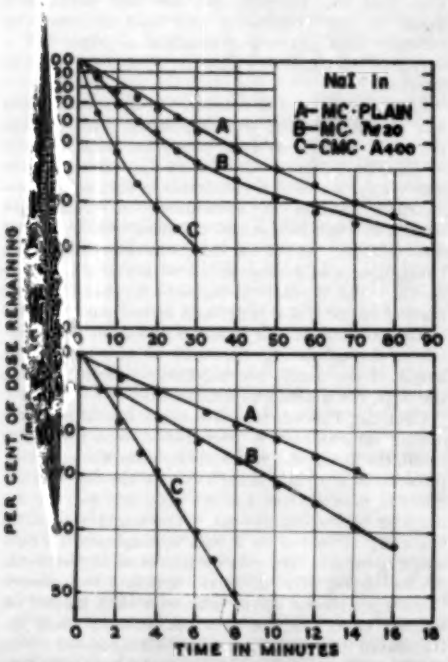

Fig. 3.—Rectal absorption curves for sodium iodide from three suppository vehicles: MC-plain, MC-Tw20-1%, CMC-A400-1%. The upper curves represent the total period of observation; the lower curves represent the initial 18-minute portion of the observed data.

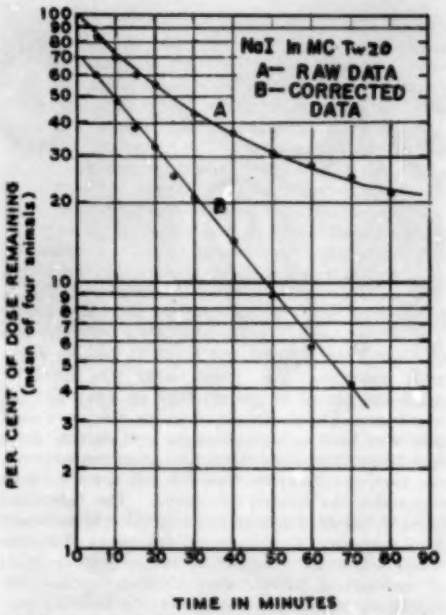

Fig. 4.—Corrected and uncorrected rectal absorption curves for sodium iodide in MC-Tw20-1%. Curve *A* represents the uncorrected data while curve *B* represents the data corrected using Eq. (8) or (9).

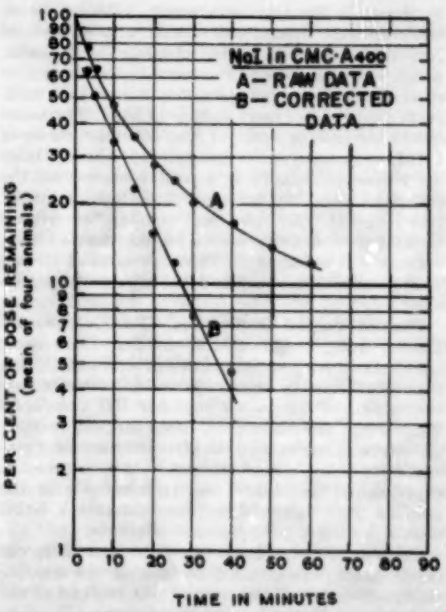

Fig. 5.—Corrected and uncorrected rectal absorption curves of sodium iodide in vehicle CMC-A400-1%. Curve *A* represents the uncorrected data while curve *B* represents the data corrected using Eq. (8) or (9).

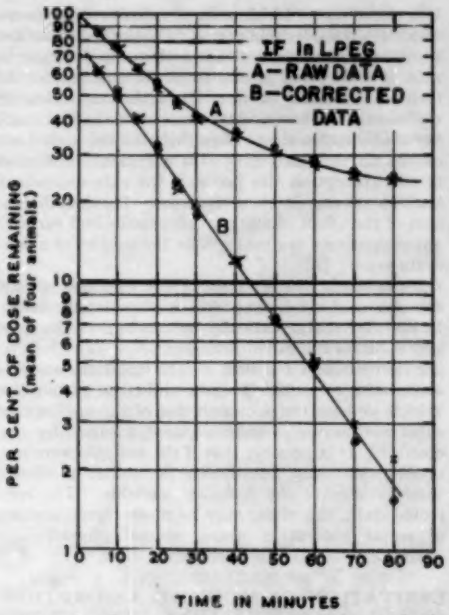

Fig. 6.—Corrected and uncorrected rectal absorption curves for iodoform in the LPEG vehicle at two different concentrations: 64 mcg./0.2 ml. (crosses), and 32 mcg./0.2 ml. (circles). Curve *A* represents the uncorrected data, while curve *B* represents the data corrected using Eq. (8) or (9).

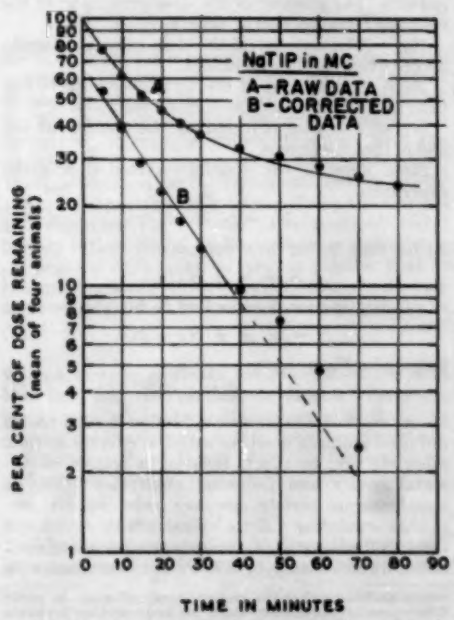

Fig. 7.—Corrected and uncorrected rectal absorption curves for the sodium salt of 2,4,6-triiodophenol in MC-plain vehicle. Curve *A*—raw data; curve *B*—corrected data.

5 microcuries per 0.2 ml. of vehicle was used: methylcellulose 4000 c. p. s.—1.5 Gm.; NaCl—*q.s.* to make isotonic; pyrophosphate buffer (0.025 *M*, pH 8.6)—*q.s.* to make 100.0 cc.

Physical Characterization of the Suppository Vehicles.—Before incorporating the labeled compounds into the suppository vehicles, the isotonicity, pH, surface tension, and viscosity were evaluated for each preparation. The vehicles were made isotonic to a 0.9% solution of sodium chloride. A thermistor resistance meter was used to check the freezing point lowering of the adjusted solution. It was impossible to evaluate directly the osmotic characteristics of the undiluted vehicle by the above freezing point method without dilution, since the vehicle separated free solid glycol on cooling. In this case, the vehicle was diluted twelvefold and data obtained from the diluted solution were used for the adjustment of the tonicity.

The viscosities of the prepared vehicles were adjusted to 750 ± 50 centipoises at 25° using a Cannon Fenske viscometer, size 500. Preparations possessing a viscosity higher than 800 were made less viscous by the addition of small volumes of isotonic, buffered solution; those giving readings lower than the accepted value were made more viscous by the addition of small amounts of appropriate thickening agent. The pH was determined with a Beckman pH meter, model G, and readjusted when necessary. All buffers were designed to possess high buffer capacity at the specified pH. Surface tension measurements were made at room temperature with a Cenco-DuNouy interfacial tensiometer.

RESULTS

A typical set of results is shown for NaI Figs. 3, 4, and 5; for IF in Fig. 6; and for NaTIP in Fig. 7.

DISCUSSION

In the development of a reproducible technique several factors were found to be of critical importance, these included: (*a*) the degree of anesthesia, which had to be deep enough to eliminate body and peristaltic movements; (*b*) geometric placement of the dose and its retention within this rectal segment by confining plugs; (*c*) the viscosity of the vehicles which prevented leakage beyond the plugs; and (*d*) the type of collimation of the scintillation counter. A scintillation counter, unless restricted by a suitable collimator, will detect any radiation emitted within an 180-degree arc. Such a counter would respond not only to the dose remaining in the rectum, but also would detect most of the absorbed dose. In order to restrict the view of the counter in so far as possible to the rectal volume segment containing the radioactive dose, several preliminary collimation studies were carried out using both cone and cylindrical type collimators attached to a scintillation counter. The isosteric patterns of each collimator were determined by detecting a known radioactive source, positioned at various vertical and horizontal coordinates relative to the crystal of the counter. It was observed that a one-inch cylindrical collimator (Fig. 2) restricted the volume segment of the animal to approximately the first four

centimeters of a rat's rectal canal; in addition, it adequately excluded from the scanned area the liver, kidneys, and other vital organs of the animal.

Studies were also conducted using a probe scintillation counter. It was believed that the small size of the sensing crystal of the probe counter inserted directly into the rectal canal would make it highly efficient in detecting the radioactive dose immediately adjacent to it, but relatively less sensitive to the radioactive molecules accumulating in surrounding tissues. Although the probe counter responded in accordance with its proposed characteristics, difficulty was experienced in placing and maintaining it in the rectum. It also scanned to a certain degree the whole animal, and for these reasons it was not used in this study.

It was found that the data followed an absorption pattern which indicated certain characteristics typical of a first-order rate process. For example, in studies using NaI in MC vehicle, it was observed that by doubling or halving the concentration of the drug, the observed time for 50% absorption was not appreciably changed. Beyond the first half-life (usually around 60% absorption), the NaI data deviate from linearity (Fig. 3, curve *A*). While this linearity through one half-life may be tacitly taken as evidence of a first-order process, the deviation should be logically explainable.

Two possible explanations perhaps account for the deviation of the NaI data from first-order: following its absorption, a portion of the NaI returns to the volume segment of the animal scanned by the counter; and in the rectal segment, physical changes occur in the vehicle which retard the rate of NaI release for absorption.

In this investigation, the experimental design is such that the volume segment scanned by the counter remains constant in respect to the total volume of the animal. The amount of absorbed drug which returns to this volume segment may also be considered a constant relative to the total amount of absorbed drug; in effect, it is a measure of the ratio of the volume segment to the total volume of the animal. These interrelationships are based on the fact that none of the drug is excreted or exhaled during the experimental procedure. It might be conjectured that the scanned area might abnormally concentrate the drug; however, the drug distribution studies contradict this contention.

The effect of the returning drug, therefore, is not a variable but a constant related to the volume fraction of the animal scanned. While this effect is constantly present, it only corrects the curvature about 5%. In order to rectify the curved portion of the plotted data to the straight line, a total correction of 20 to 5% is required.

A second explanation of the curvature could be based on an assumption that diffusion of the drug, within the vehicle, to the absorption site becomes the rate limiting step which, in turn, would modify the rate of absorption. When vehicles are formulated with surfactants, NaI is absorbed at a significantly faster rate than in the plain vehicles. The faster absorption apparent in these systems results in greater curvature. These results, shown in Fig. 3 (curves *B* and *C*), are in accordance with the assumption that the limiting factor is the diffusion of the drug to the absorption site. In the slow absorbing systems the drug can diffuse to the absorption site at a rate which maintains first-order absorption for a considerable period. With the surfactant systems, one might postulate that the increase in rate of absorption is due to slight damage to the membrane barrier or that the surfactant "cleans" the membrane barrier and thus increases the number of absorption sites. In either case the surfactant causes an increased rate of absorption. Diffusion to the absorption site becomes the rate dependent variable sooner in these systems. Further discussion of the effect of anionic compounds and surfactant suppository systems will be the subject of a separate report (26).

Fortunately the analysis of the rate process for experimental conditions used in these studies may be handled mathematically by analogy to the general solution for radial diffusion (27). The following derivation is for such a rate equation, and includes the correction for the diffusion within the vehicle and for the accumulation of the radioactive substance in the volume segment scanned by the counter. It is possible that if the animals were not under such deep anesthesia, peristaltic processes would eliminate the diffusion variable. The corrected data, therefore, may be more representative of rectal absorption under normal physiological conditions than are the uncorrected data.

DERIVATION OF MODIFIED ABSORPTION EQUATION

N_o = The total dose administered (detected by the scintillation counter). N = The dose detected by the counter at time, t. f = The volume fraction of the animal being detected by the counter modified by local variations in drug distribution.

N_r = The amount of the dose remaining in the confined rectal section at time, t.

N_a = The amount of the dose remaining unabsorbed at end of the experiment.

N_b = The amount of the total dose absorbed at time t, and distributed throughout the body.

N_f = The dose detected by the counter at the end of the experiment.

Since none of the dose is excreted during the experiment,

$$N_o = N_r + N_b \qquad (1)$$

At any time during the experiment,

$$N = N_r + f(N_b), \qquad (2)$$

or substituting for N according to Eq. (1), we have

$$N = N_r + f(N_o - N_r). \qquad (3)$$

Solving for N_r, we have:

$$N_r = [N - f(N_o)]/(1 - f). \qquad (4)$$

In our work the experimental design is such that a diffusion gradient is set up within the rectal section. Since we are, in effect, isolating a section of the rectal cavity and following absorption from its membraneous surface, we can refer to our section as simulating a finite cylinder with sealed end faces, diffusion out of the cylinder occurring in a radial direction only.[3] We are not concerned with

[3] It is obvious that the rectum does not meet the exact dimensions of a cylinder; since the cross-section probably resembles an ellipse in shape. It can be shown, however, that such considerations merely change the absolute values of the constants in the final equation. See Jost, W., "Diffusion in Solids, Liquids, Gases," Academic Press Inc., N. Y., N. Y., 1952, p. 35.

the concentration of the drug at any point along the radius of the cylinder; instead, we are following the average drug concentration, defined above as N_r with an initial concentration N_o and a final concentration relating to the total unabsorbed drug, N_u. Radial diffusion according to these conditions has been solved by Dünwald and Wagner (27) using Fourier analysis; the solution is:

$$(N_r - N_u)/(N_o - N_u) = \sum_{i=1}^{\infty} 4/X_i^2 \exp\left[-(X_i^2)^2 Dt/r_o^2\right]. \quad (5)$$

Here r_o represents the radius of the rectal cylindrical section, D is the diffusion coefficient, and the X_i are the roots of the equation $J_0(X) = 0$, where $J_0(X)$ is the Bessel-function of the zero-th order and second kind (X_i = 2.405, 8.654, 11.792,—). From an estimation of r_o and D it may be shown that for t>3 min., the first term of the series is a good approximation for the complete equation, therefore:

$$(N_r - N_u)/(N_o - N_u) = 4/(2.405)^2 \cdot \exp\left[-(2.405)^2 D/(r_o)^2 \cdot t\right] \quad (6)$$

or

$$(\log N_r - N_u)/(N_o - N_u) = -kt + (\log C) \quad (6a)$$

Where $k = r_o^2/(2.303)(2.405)^2 D$; $C = 0.7$[4]

Since our counter detects N instead of N_r we must substitute for the latter according to Eq. (4).

$$\log \frac{[N - f(N_o)/(1-f)] - N_u}{N_o - N_u} = -kt \quad (7)$$

Rearranging and collecting terms we arrive at:

$$\log \frac{N - N_u - f(N_o - N_u)}{(1-f)(N_o - N_u)} = -kt \quad (8)$$

We have spoken of a correction term relating to the accumulation of the drug in the volume segment scanned by the counter. It should be noted that Eq. 8 contains no variable function relating to this correction; it may be shown, however, that the constant, f, is the term covering this accumulation. This phenomenon has been demonstrated repeatedly at various time intervals by sacrificing the animals and removing the rectum. The removed rectal portion is then placed on the counter in a geometrical position duplicating as near as possible that in the intact animal. Such a procedure results directly in an evaluation of N_r and by rearranging Eq. 1 to

$$N_o - N_r = N_b.$$

we can directly evaluate the amount of the dose absorbed, N_b. By placing the animal (minus the rectal portion) back in its proper geometry, the counter detects only the dose in the volume segment. The latter is N of Eq. 2 where N_r becomes zero, and we then have

$$N/N_b = f.$$

Under our experimental conditions, the constant, f, thus determined is well approximated by the value -0.05. It does not vary to a large extent even when following different drugs or markedly different types of formulations.

Taking a typical set of experimental results, the value scanned by the counter at the end of the experiments N_f where: $N_f = N$ (90 min.) = 0.25; N_u = 0.20; and f = 0.05. Substituting these values into Eq. (8), we have

$$\text{Log}\,[N - 0.05(1-0.20) - 0.20]/(1 - 0.20)(1 - 0.05) = -kt$$

or

$$\text{Log}\,N - 0.24/0.76 = -kt.$$

This correction rectifies the raw data to a straight line function which holds over three to four drug half-lives.

It should be noted that a further simplification can be made in Eq. 8 in light of the detailed results given for the typical case listed above. The correction function is nicely approximated within the accuracy of our animal data by using the last determined experimental count detected by the counter on the intact animal, *viz.* $- N_f$. In the above example, Eq. 8 would result in a correction function of

$$\log N - N_f/N_o - N_f = -kt$$

or

$$\log [N - 0.25]/0.75 = -kt.$$

Any procedure which reduces the research workers exposure to gamma radiation is a valuable one. The use of Eq. 9 in place of Eq. 8 is well jutified, since it eliminates the necessity of additional close contact with the radioactive source during the excising of the rectum.

The derivation does not include any mathematical consideration of the rate process for drug transfer across the rectal membrane. In general, we believe that the absorption rate process is much faster than the diffusion process, i.e., $k_{abs.} >> k_{diff.}$

Although the apparent rate constant, k, for Eq. 9 is complex in nature, including: diffusion, absorption, and differences in formulation, it is extremely useful in expressing our data and making comparative analysis of different suppository systems.

Confirmation of the Modified Absorption Equation.—Curve *A* in Figs. 4 and 5 represents a plot of the uncorrected data; Curve *B* of these figures is a plot of the corrected data as obtained using Eq. 8, or 9. Discrepancies between the absorption half-lives for the raw and corrected data can be attributed to several causes. Since a curvature is present to a slight degree even in the early data, the 50% absorption time from the raw data will tend towards a longer $t^1/_2$ than the corrected data. The derivations of Eq. 9 implicitly assumes that the diffusion coefficient did not change with relative changes in drug concentration, that the ideal diffusion process was undisturbed by peristaltic processes, or that mixing due to temperature gradients did not take place.

Although the data presented thus far are only for NaI studies, it was found also that IF and TIP studies could be analyzed in the same manner. In each case, the data conformed to criteria for a pseudo-first-order absorption rate process. For example, when IF was used in the LPEG vehicle in concentrations of 33 mcg./0.2 ml. and 64 mcg./0.2 ml., the data expressed in Fig. 6 is obtained. When

[4] If we consider the rectum as possessing a different geometric shape, the absolute values of K and C change. For example, for the first term of the series: C = 0.7 for a cylinder, and C = 0.9 for a slab. The value of C only modifies our intercept. Recognizing that our equation is a good approximation only at t > 3 min we can neglect the second term on the right hand side of Eq. (6a).

the concentration of IF is doubled in LPEG, no apparent difference is observed in the absorption rate. Data obtained for TIP in vehicle, as is shown in Fig. 7, also meet the criteria for the pseudo-first-order absorption process.

The deviations about the straight line drawn for the corrected data (curve *B*) are more apparent than real; the lower per cent values amplify the apparent error.

A definite advantage of the derivation is that it arrives at a pseudo-first order equation as a natural consequence of accounting for the diffusion phenomenon. Although it fails mathematically to consider the rate process across the rectal membrane, there is good indication that absorption rate process of the drug is much faster than the diffusion process in the vehicle, that is, $k_{abs} >> k_{diff.}$ In general, the derivation corrects the rectal absorption data through two to four half-lives.

The form of the derivation of the absorption equation and its subsequent confirmation appears to justify the use of first-order kinetic analysis of the experimental results. Yet this procedure is not free from some uncontrolled variables. The exact correction factor which may be applied to rectify the data appears to be somewhat dependent on: the time at which the experiment is arbitrarily conducted; the character of the vehicle, and the rate of absorption therefrom; the possible variation in the amount of drug returning to the field of vision of the counter, due to variation in the amount of urine retained in the bladder of each of the individual animals studied; and other variables. While these variables do not deter from the essential validity of the foregoing presentation they do influence slightly the absolute value of the absorption half-lives obtained by analysis of these results.

Therefore, it appears more logical to analyze our data on the basis of the initial portion of the curve, where each of the above-discussed concomitant events are yet insignificant in their effect; as is shown, for example, in the lower set of curves in Fig. 3.

SUMMARY

1. A micro method is reported for the preparation of I-131 labeled iodoform, and 2,4,6-triiodophenol at a level of activity of approximately 150 to 200 μc./mg.

2. A highly reproducible animal procedure was developed for the continuous external detection of a gamma-emitting dose in the rectum of an anesthetized rat.

3. A mathematical expression was derived which corrects the activity detected by the counter for the effect of diffusion within the vehicle, and for the accumulation of the absorbed drug in the scanned volume segment of the animal.

4. Diffusion of the drug to the rectal absorption site appears to be the rate controlling step in fast absorbing systems.

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The Kinetics of Rectal Absorption II.*

The Absorption of Anions

By SIDNEY RIEGELMAN and WILFRED CROWELL†

The rectal absorption rates of sodium iodide and the sodium salt of 2,4,6-triiodophenol in suppository bases at various pH values and in the presence of surface-active agents and polyethylene glycol additives were determined by continuous external detection at the site of insertion. The rate of sodium iodide absorption is accelerated in the presence of surfactants and appears to be proportional to the surface tension lowering and the peptizing action of their surface active components. The rate of absorption of the phenolate is retarded by the presence of surfactants. Hydrolysis and subsequent solubilization of the free phenol is postulated to explain this effect.

IN THE FIRST PAPER of this series (1), a method (using a female rat) was reported for the direct and continuous external detection of the rate of rectal absorption of drugs from suppository vehicles. The rate of absorption of sodium iodide, iodoform, and 2,4,6-triiodophenol tagged with iodine-131 could be followed by virtue of the gamma emission from the radioactive source which was detected by a scintillation counter in conjunction with an autoscaler. The absorption data appeared to follow a pseudo-first order rate process when corrected for the effect of two concomitant events. The drug is continuously returning to the environments of the absorption site and is detected there by the counter. Also within the confined rectal segment to which the suppository was limited, the rate limiting step in the overall absorption process is assumed to be the diffusion of the drug within the vehicle. An equation was presented which rectified the data for analysis to conform to a pseudo-first order rate process. An alternative to the use of the corrected data is the analysis of the initial portion of the uncorrected data where each of the factors causing deviation would be at a minimum. Analysis of the initial data probably results in an absorption half-life which is slightly too long, due to the small amount of curvature present even in the earliest points. While the absolute values of the specific rate constants may differ with each method of analysis, the relative comparison of the data by either method results in identical conclusions. For this reason, the simpler method of analysis using the initial data has been adopted in this report.

In the past several years, studies have been conducted (2, 3) which report on the absorption of sodium iodide from common type suppository bases. One such study (3) has utilized radioiodide as the means of detection. The authors based their conclusions on results of analysis of peripheral level of iodide in the blood, thyroid, or in the urine. While certain information can be obtained by these methods, they appear to be poorly reproducible and to give little indication of the details of the rate process directly at the site of insertion. Apparently no studies have been conducted on the effects of surface-active agents on the rate of rectal absorption, even though several nonionic surface-active agents have been utilized as major constituents of several suppository bases (4, 5).

Polyethylene glycols have been utilized quite extensively in commercial suppositories. Yet, inconsistent and contradictory results (6–10) have been obtained from studies comparing their rate of drug release to that obtained by cocoa butter or by glycerinated gelatin. Once again, these studies were restricted to methods of analysis which detect events distant from the site of absorption; thus the conclusions drawn from these results may be clouded by the number of intervening biological processes. Furthermore such studies would probably be unable to detect differences caused by complexing of the drug with the polyoxyethylene chain, as has been proposed by Higuchi and Lach (11).

The present communication presents the results of the evaluation of the absorption rate of sodium iodide (NaI) and the sodium salt of 2,4,6-triiodophenol (NaTIP) at various pH values and in the presence of surface-active agents and polyethylene glycol additives.

RESULTS AND DISCUSSION

Both sodium iodide and triiodophenol can exist as anions at certain hydrogen ion concentrations.

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This paper is based in part on a thesis submitted to the Graduate School of the University of California by W. J. Crowell in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
† Parke Davis Fellow. Present Address: University of Southern California School of Pharmacy, Los Angeles.

Sodium iodide exists as an anion at all pH values which can be reasonably studied in the rectum and possesses high water solubility. In contrast, triiodophenol is a weak acid of very low solubility. The acid dissociation constant of the compound was reported by Krahl and Clowes (12) and was confirmed by the authors to be 6.6. The solubility of the compound in an unbuffered solution containing 5% of polyethylene glycol 600 was found to be 300 mcg. per 100 ml. At pH 8.6, triiodophenol would be distributed 99.01% as the anion and 0.99% as the free acid. An isotonic, liquid polyethylene glycol 6000 vehicle (LPEG) containing 59% of a purified sample of polymer was used in these studies to eliminate the effects of such variables as: water transfer due to the nonisotonic nature of polyethylene glycol suppositories, and/or the solution of the solid phase by the transferred water. Due to the large molecular weight of the glycol, one can assume that the compound is very slowly, if at all, absorbed; therefore, the isotonic vehicle can be expected to remain undiluted during the period of study. The dissociation of the triiodophenol in such a semiaqueous solvent system will be reduced relative to an aqueous system. The dissociation constant was found to be 8.4 in this vehicle. In order to convert the free acid to the same proportion of anion as used in the aqueous systems, this vehicle was adjusted to pH 10.7 with a sodium hydroxide-sodium borate buffer.

The results obtained with sodium iodide in the vehicles studied are summarized in Table I and several of these results are depicted in detail in Fig. 1. As one might anticipate, at the same level of viscosity, a change from methylcellulose to carboxymethylcellulose does not appreciably alter the rate of absorption of sodium iodide. It was necessary to substitute the latter agent in several of the vehicles studied due to the coacervation of methylcellulose in the presence of certain surface-active agents.

TABLE I.—COMPARISON OF THE FIFTY PER CENT ABSORPTION TIMES OF VEHICLES CONTAINING SODIUM IODIDE

Type	Vehicle Additive	Surface Tension (dynes/cm.)	50% Absorption Times ($t^1/_2$)
MC	Plain	52	33.1 ± 0.8
CMC	Plain	52	31.4 ± 0.5
LPEG	Plain	46	29.5 ± 1.3
MC	Tw20-1%	34	21.5 ± 0.6
MC	Tw20-5%	34	22.8 ± 0.6
CMC	Tw20-1%	33	22.2 ± 0.4
CMC	SLS-1%	30	8.9 ± 0.2
CMC	A400-1%	30	8.4 ± 0.3

The addition of surface-active agents to these systems markedly accelerated the rate of absorption of the sodium iodide when compared to the rate in their absence. Surface-active agents are frequently said to cause protein denaturation and may actually damage the integrity of the mucous membrane lining of the rectum. Such an explanation might be presented for the increased rate of absorption of iodide ions in the presence of surfactants. Yet, identical studies conducted on sodium triiodophenolate (NaTIP) in the presence of the same group of surfactants indicate that this ion is actually retarded in its rate of absorption relative to the rate in the absence of surface-active agents. It is difficult to visualize how the surfactant can damage the mucous membrane in such a way that iodide ions can penetrate, yet not cause an increased rate of absorption of the phenolate ion.

Since the segment from which absorption is taking place is held constant by the confining end plugs, the action of the surface-active agents on the rate of drug absorption cannot be attributed to the spreading of the vehicles over a larger rectal segment. The observed differences in time for 50% absorption of NaI in the presence of Tween® 20 (Tw20), sodium lauryl sulfate (SLS) and Antarox® A400 (A400)[1] appear to correlate with the relative

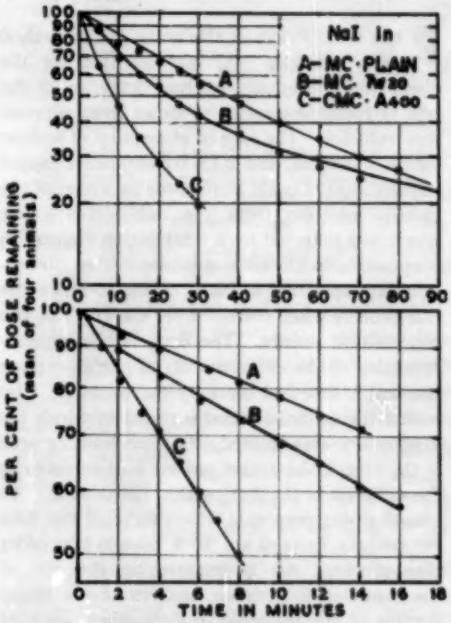

Fig. 1.—Rectal absorption curves for sodium iodide from three suppository vehicles: MC-plain, MC-Tw20-1%, and CMC-A400-1%. The upper curves represent the total period of observation; the lower curves represent the initial eighteen-minute portion of the observed data.

surface tension lowering of the respective vehicles. Since the rectal membrane is covered with a relatively continuous mucous blanket, the acceleration of NaI absorption might well be ascribed to the mucous peptizing action of the vehicle ingredients. As a result of this cleansing action, additional pore spaces could be made available to the iodide ions, thus facilitating their movement across the membrane barrier.

It is interesting to note the data obtained with sodium iodide dissolved in the LPEG vehicle are also in accord with the above peptizing theory.

[1] This compound is a polyethylene glycol ether of nonyl phenol and is now sold under the trade name of Igepal® CO-630.

The cleansing or peptizing capacity of surfactants is not well represented by comparison of the reduction in surface tension of the solution. Several physical tests are required to evaluate the detersive activity of surfactants (13), however, such studies indicate that Tw20 is less effective than SLS or A400. The latter two agents appear to be equally effective in their detergent action. A similar relationship was found in the relative effectiveness in modifying the rate of absorption of NaI.

No doubt, without further experimentation, any theory put forward to explain the effect of surfactants will be controversial; nevertheless the fact remains that the addition of small amounts of these additives strikingly increases the rate of absorption of the iodide ion from the formulations studied. It appears that they accelerate the rate fourfold to fivefold.

Table II and Fig. 2 appropriately summarize the data obtained for NaTIP in the suppository systems studied. Once again the data confirm the postulate that methylcellulose and carboxymethylcellulose are interchangeable in the vehicles with little or no effect on the rate of absorption. Thirty-two milligrams of NaTIP were present per 100 ml. of the vehicles. Therefore at pH 8.6, where 99% of the compound was in the anion form, the vehicle approached saturation in respect to the free acid (HTIP). At pH 10, however, only 0.03% of the total drug present was in the free acid form, making the vehicle approximately 3% saturated. Even though there is a thirtyfold change in HTIP concentration between 8.6 and 10.0, a relatively insignificant change occurs in the absorption half-life of the drug.

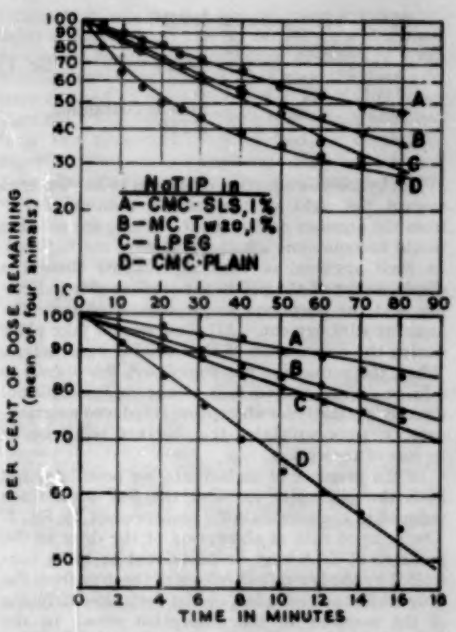

Fig. 2.—Rectal absorption curves for sodium triiodophenolate from four suppository vehicles: CMC-SLS-1%, MC-Tw20-1%, LPEG, and CMC-plain. The upper curves represent the total period of observation; and the lower curves represent the initial eighteen-minute portion of the observed data.

TABLE II.—COMPARISON OF THE FIFTY PER CENT ABSORPTION TIMES OF VEHICLES CONTAINING SODIUM TRIIODOPHENOLATE

Vehicle		Surface Tension (dynes/cm.)	50% Absorption Times ($t^1/_2$)	pH
Type	Additive			
MC	Plain	52	17.3 ± 1.4	8.6
CMC	Plain	52	18.6 ± 0.2	8.6
MC	Plain	55	19.9 ± 0.4	10.0
LPEG	Plain	46	42.6 ± 0.4	10.7
CMC	SLS-0.25%	33	24.2 ± 0.3	8.6
CMC	SLS-1%	33	59.3 ± 1.7	8.6
MC	Tw20-1%	34	40.3 ± 1.2	8.6
MC	Tw20-5%	34	41.8 ± 0.8	8.6

The time for 50% absorption of NaI in the absence of surfactants is significantly greater than that for NaTIP even though the molecular volume of the iodide ion is appreciably smaller than the phenolate ion. The reverse might have been expected since both the diffusion and absorption processes are known to be related to molecular volume characteristics. Theoretically, there are several factors which may account for the rate differences. Even though the absorption of NaI possesses first-order characteristics over a short range of concentrations, its absorption might be sufficiently altered to give a slightly shorter half-life if used in reduced concentrations. A more plausible suggestion is that a portion of the phenolate ion is converted into the free phenol at the absorption site during the transfer of ions between intracellular and extracellular fluids. Since both HTIP and the NaTIP ion might be absorbed independently, the observed absorption rate may be the sum of the individual absorption rates.

While surface-active agents accelerate the absorption of NaI, the data presented in Table II and in Fig. 2 clearly indicate that the reverse is found for NaTIP systems. One explanation of the slow rate of absorption might be related to the observation of Higuchi and co-workers (11), who have pointed to the complexing of certain phenols with long chain polyoxyethylene polymers. One might conclude from their work that either the phenolate ion or the free phenol complexes with the polyoxyethylene moiety in the micelles, or directly as in the case of the LPEG vehicles.

In contrast to Tw20 and A400, SLS possesses no polyoxyethylene chains, but instead has an anionic polar sulfate group; nevertheless, it markedly retards NaTIP absorption. Since micelles are present in the SLS systems studied, solubilization of the drug within the hydrocarbon portion of the micelle might be postulated. This kind of solubilization would require interaction of the anionic triiodophenolate ions with the anionic surfactant ions. Such an occurrence is unlikely since it would be opposed by the coulombic repulsive forces between these ions. However, the surfactant micelle could solubilize the undissociated triiodophenol.

In the presence of surface-active agents, the following equation may indicate the interrelationship of the surfactant to the triiodophenol:

$$H^+ + TIP^- \rightleftharpoons HTIP$$
$$\Big\downarrow SAA \qquad \text{(Eq. 1)}$$
$$\text{Micelle containing HTIP}$$

The hydrolysis equation continues to be displaced toward the right as the micelle removes HTIP from the aqueous environment. Thus, the solution would become more alkaline if it were not buffered. In such systems, *in vitro* experiments showed a displacement of the pH to the alkaline side indicating that the phenolate ion was removed from the aqueous environment. All these events take place during the preparation of the dose before insertion. When the surfactant has complexed the phenol to a large degree, the amount of uncomplexed phenol directly available for absorption is reduced commensurably, thus explaining the observed reduction in its rate of absorption.

In the presence of surfactants, we postulate that both the phenolate ion and the free phenol are reduced in their availability as expressed by Eq. 1. The reduced rate of absorption of the drug in the presence of SAA may be postulated as being controlled by the low rate of release of the drug from the drug-surfactant complex, or the very slow diffusion of the complex to the absorption sites. In the absence of the surfactant, both the phenol molecule and the phenolate ion are free for diffusion and absorption; their ratio would be controlled by the pH of the media. As mentioned previously, no significant difference is observed in the absorption rate to NaTIP at pH 10.0 and 8.6. The phenolate ion upon reaching the absorption site could be partly absorbed as the ion, and partly converted at the vehicle membrane interface to the free phenol; thus transferring across the rectal epithelial cells.

EXPERIMENTAL

The radiological procedures and equipment, pharmacological and counting procedures, the procedure of synthesis of TIP, the method of determining radiochemical purity of the TIP, the assay of the radioactivity of the labeled compound, and the physical characterization of the suppository vehicles have been described previously (1).

Reagents.—The reagents used in this study which were not reviewed before (1) include: sodium lauryl sulfate (Dupont), which was twice recrystallized from boiling alcohol and washed with ether. The purity of the dried compound was determined by dissolving a sample in distilled water and allowing it to pass through an acid activated cation exchange resin column (Dowex® 50) until the filtrate reached a pH of approximately 7. The combined washings were then titrated with a standardized solution of potassium hydroxide, using phenolphthalein as the indicator. The neutralization equivalent thus obtained from the free acid, lauryl sulfuric acid, was found to be 292.7.

Formulas for Suppository Vehicles.—In the NaI studies, 5 μc. of NaI-131 was added for each 0.2 ml. of vehicle used. The basic formula for the methylcellulose vehicle (MC) or the carboxymethylcellulose vehicle (CMC) follows:

Methylcellulose 4000 c. p. s., or sodium carboxymethylcellulose, high viscosity type	1.5 Gm.
Sodium iodide	0.167 Gm.
Sodium bisulfite	0.050 Gm.
Sodium chloride	*q.s.* to make isotonic
Phosphate buffer (0.050 *M*, pH 7.4)	*q.s.* to make 100.0 ml.

The above formula includes the sodium bisulfite to maintain the iodide in the reduced state. The surface-active agents were added to the above formulations in the required amounts (1 or 5%) and the vehicle readjusted for tonicity and viscosity. The isotonic liquid polyethylene glycol vehicle (LPEG) was designed to contain a large amount of purified polyethylene glycol 6000 to act as a solvent, an agent to increase the viscosity, and to balance the tonicity of the vehicle. The formula included:

Polyethylene glycol 6000	59.0 Gm.
Polyethylene glycol 600	5.0 Gm.
Sodium iodide	0.167 Gm.
Sodium bisulfite	0.050 Gm.
Phosphate buffer (0.05 *M*, pH 7.8)	*q.s.* to make 100.0 ml.

Triiodophenol was added to a buffered methylcellulose (MC), or carboxymethylcellulose (CMC), vehicle in order to form the sodium phenolate. Once again 5 μc. per 0.2 ml. of vehicle was used.

Methylcellulose, 4000 c. p. s., or carboxymethylcellulose high visc. type	1.5 Gm.
Polyethylene glycol 600	5.0 ml.
Pyrophosphate buffer (0.050 *M*, pH 8.6), or borate buffer U. S. P. pH 10.0	*q.s.* to make 100.0 ml.

The surface-active agents were added to the above formulations in the required amounts (1 and 5%) and the vehicles were readjusted for tonicity and viscosity. The isotonic, liquid polyethylene glycol vehicle (LPEG) used for triiodophenol is listed below:

Polyethylene glycol 6000	59.0 Gm.
Polyethylene glycol 600	5.0 Gm.
Boric acid	0.33 Gm.
Sodium borate	0.13 Gm.
Distilled water (pH 10.7)	*q.s.* to make 100.0 ml.

Design of Experiments and Statistical Analyses—Suppository vehicles were designed to possess only one variable such as the absence or presence of a surface-active agent during each experiment. The effect of such a variable on the rate of rectal absorption of the radio tagged drugs was then assayed by tracer techniques using an experimental series of four animals. In order to minimize inaccuracies in counting, the level of radioactivity used in each experiment resulted in an initial counting rate of 400 to 500 counts per second. At the end of the experiment, the counting rate rarely fell below 75 counts per second, while the background count was approximately 3 to 4 counts per second.

The data from each experimental series were collected in tabular form with certain mean per cent values converted to log equivalents, which were used in the analysis of the data by linear regression methods adapted from Snedecor (14). For data involving rapid absorption, the values for the slope and standard error of the slope were based on log

equivalent for the first eight minutes of each experimental series; for slow absorption, for all other absorption studies, on data collected for the first eighteen minutes. Time for 50% of the dose to be absorbed from each vehicle was determined by substituting the values of the slope and standard error of the slope into the following equation:

$$t^1/_2 \text{ (in mins.)} = 0.301/(\text{slope} \pm 0.301/\text{standard error of the slope}) \quad \text{(Eq. 2)}$$

Solubilization Studies.—The solubilization of TIP in various aqueous systems was determined by the following nephelometric procedure. Approximately 100 mg. of the compound was dissolved in sufficient PEG 600 to make a stock solution containing 1 mg. of solute per 1000 mg. of solvent. Aliquots of the stock solution were weighed into 100-ml. tared volumetric flasks and then diluted to volume with isotonic solutions or isotonic buffered solutions containing the surface-active agent. After mixing, the degree of light scattering for each system was detected with a Fischer Nefluoro-Photometer and the per cent transmission was plotted against solute concentration. By extrapolation of the plotted points to solvent transmission, a numerical value was obtained for the amount of solute solubilized in each system.

Determination of the Apparent Dissociation Constant of Triiodophenol in PEG Vehicles.—The determination was carried out at room temperature using a Beckman pH meter, Model G, and a 1-ml. semimicro buret. Five-milliliter aliquots of PEG vehicle containing 0.02 millimole of the free acid were titrated to neutrality with 0.1*N* NaOH in an identical PEG vehicle. The pH readings obtained from this procedure were plotted on centimeter paper against the microliters of alkali added. From the titration curves thus constructed, the pK was evaluated to be 8.4 ± 0.1.

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The Kinetics of Rectal Absorption III.*

The Absorption of Undissociated Molecules

By SIDNEY RIEGELMAN and WILFRED J. CROWELL†

A study of the rectal absorption of iodide-131 tagged iodoform and 2,4,6-triiodophenol, at pH's where the drug is undissociated, was carried out in liquid semi-aqueous isotonic vehicles containing the drug in suspension, in true solution, and in solution in surfactant micelles. First order analysis of the results indicated that the drugs are best absorbed from true solutions in water, or from water suspensions of the drug. Surface-active agents and polyoxyethylene polymers markedly retard the rate of absorption. The relative particle size of the suspended drug influenced the absorption rate. Solution of the drugs in solid polyethylene glycol and oleaginous bases resulted in very prolonged absorption times. Head count studies (simulating continuous blood level studies) and the data on the tissue distribution of the compounds are also presented.

OIL-SOLUBLE, undissociated compounds have been used frequently as test drugs by workers studying the factors affecting the rate of absorption of drugs from suppository bases. However, probably due to the method for detection of the drug available to these workers, only conditions producing differences of relatively large magnitude have been able to be detected. One of the most important of these methods has been the analysis of the concentration of the compound in the blood stream. While this method results in valuable information pertinent to that one drug, such studies are confounded by biotransformations of the drug and by the potential difference in the affinities of the tissues for the compound at varying rates of absorption. The results, therefore, have been very difficult to generalize to other compounds, even in the same vehicle. Unless there is a direct, proven relationship between the rate of absorption at the

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† Parke Davis Fellow. Present address: University of Southern California School of Pharmacy, Los Angeles.

rectal site and the blood level, it is difficult to draw general conclusions. By the use of a direct radiological method of detection of the events taking place at the site of absorption, this present series of studies (1, 2) has been able to detect relatively subtle differences in the rate of absorption from suppository vehicles.

This communication reports the results obtained by studying the absorption of two radiotagged, undissociated molecules: 2,4,6-triiodophenol (HTIP), and iodoform (IF) from the rectal passage of a female rat. Liquid isotonic semi-aqueous vehicles have been formulated containing the drugs in suspension, in true solution in water and in polyethylene glycol, and micellularly solubilized by surface-active agents. Solid polyethylene glycol and cocoa butter type bases have been studied in an attempt to correlate such data with the results of other workers in the field. In addition, this communication includes data on the total tissue distribution of the drugs and head counts (simulating continuous blood level studies) on the two undissociated molecules as well as sodium iodide. It is now possible to draw some comparisons and generalizations from the studies relative to the work done by other techniques.

RESULTS AND DISCUSSION

It is essential for these studies that extraneous iodide-131 be removed or reduced to an absolute minimum in the samples of iodoform and triiodophenol. During the synthesis of the compounds, several additional steps were taken to remove all traces of iodide-131. The purity of both compounds was evaluated by paper chromatography combined with radioautographic techniques. Radio-tagged iodoform samples were found to contain one to two% radioactive iodide impurities. If the iodoform were decomposed to a large extent into iodide, the rate of absorption of the compound should follow the pattern of results obtained with that ion. The significant difference in results between the rectal absorption rates of IF and NaI-131 appears to confirm the postulate that the compound is relatively free of trace iodides and is not broken down to iodide prior to absorption. Radioactive triiodophenol was found to be essentially free of radioactive iodide impurities. Both compounds were stored in a nonaqueous solution composed of undiluted polyethylene glycol 600. This measure was necessary to prevent contamination of the area, since both compounds sublimed when stored as solids. In the majority of experiments, the radio-tagged samples were used for only a period of seven days or less in order to stay within one radioactive half-life of the iodide.

The water saturation concentration for iodoform in the plain methylcellulose vehicle was found to be 8 mg. %. In the vehicles reported in Table I, iodoform was present to the extent of 32 mg. %. Therefore, the compound was present as a suspended solid phase, exceeding its solubility in the plain MC vehicle by fourfold. However, when the surface-active agents were added to the MC vehicle or when the isotonic liquid polyethylene glycol (LPEG) vehicle was studied, the iodoform was completely dissolved.

TABLE I.—FIFTY PER CENT ABSORPTION TIMES FOR TRIIODOPHENOL-IODOFORM FROM VARIOUS VEHICLES

Vehicle		50% Absorption Time (hrs)	
Type	Additive	IF	HTIP
MC	plain	13.3 ± 0.5	35.0 ± 0.8[a]
MC	Tw 20-0.25%	.....	28.5 ± 0.6
MC	Tw 20-1%	24.7 ± 1.2	39.7 ± 0.5
MC	Tw 20-5%	25.1 ± 0.4	44.9 ± 0.8
LPEG	.....	23.2 ± 0.4	43.8 ± 1.3[b]
MC	SLS-0.25%	.....	49.6 ± 0.9
MC	SLS-1%	.....	73.3 ± 1.7

[a] All MC-HTIP vehicles were adjusted to pH 4.6.
[b] All LPEG-HTIP vehicles were adjusted to pH 5.9.

Triiodophenol has been found to possess a pK_a of 6.6 in water (3), and 8.4 in the LPEG vehicle. Therefore, when dispersed in a MC vehicle buffered to pH 4.6, the triiodophenol is present to the extent of 99% in the free acid form (HTIP). In the LPEG vehicle, the drug was buffered to pH 5.9 in order to assure that greater than 99% of the amount present was in the undissociated form. In the studies reported in Table I, the concentration of triiodophenol was maintained at 32 mg. %. The solubility of the compound in the MC vehicle was found to be 300 mcg. %; the compound, thereby, was present as a suspended phase in the MC vehicle exceeding the solubility by approximately one hundredfold. The solubility was markedly increased when the surface-active agents were added to the MC vehicle containing 32 mg. % of triiodophenol. When 0.25% of sodium lauryl sulfate or Tween®20 were included, the HTIP only slightly exceeded the saturation concentration of the two vehicles, producing a slight amount of turbidity. The compound was completely in solution in all the other vehicles included.

In Fig. 1, curve C represents IF in the plain MC vehicle where it is in suspension; curve B, IF in solution in MC-Tw 20 1% vehicle; and curve C, IF in solution in the LPEG vehicle. Although the aqueous systems contain the drug in excess over water solubility, the rate of absorption is greater than in the Tw 20 and LPEG systems. It is interesting to observe that the rates of absorption from the two latter vehicles are very similar even though the LPEG vehicle contains many more equivalents of oxyethylene groups than the Tw 20 vehicle.

Figure 2 represents HTIP in an aqueous MC suspension—curve D, and in the presence of various concentrations of Tw 20 and SLS—curves A to C. The systems containing SLS are more effective in retarding the drug absorption than are the Tw 20 systems. A 0.25% concentration of SLS produced a slower rate of HTIP absorption than did a 5% concentration of Tw 20. The drug is micellularly solubilized by both surfactants. From the studies of Higuchi and co-worker (4), we can assume interaction of the phenol with polyoxyethylene chains of Tw 20. Practically nothing is known of the type of interaction between the HTIP and the lauryl

sulfate ions; yet from the above data we must presume that the latter type of complexing is greater than the former, when evaluating on the basis of drug release.

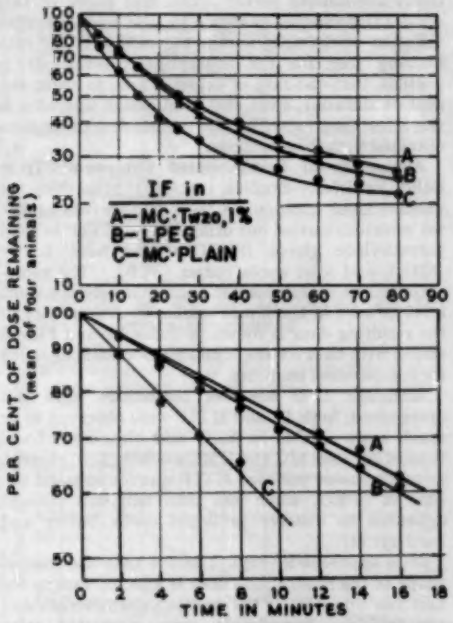

Fig. 1.—Rectal absorption curves for iodoform from three suppository vehicles. Upper curves represent total period of observation; lower curves represent initial expanded portion of observed data.

Comparison of the HTIP absorption rates at the 0.25% level of the respective SAA indicates an anomaly in the results. The 0.25% Tw 20 system caused a slight increase in the rate of absorption when compared to the aqueous suspension; while the SLS 0.25% system produced a marked retardation. Both of these vehicles contained a small excess of the drug, as compared to the aqueous MC suspension; the large increase in water solubility being due to the presence of micelles in both solutions. The rate of solution of the drug from the solid interface, as well as its rate of release from the drug-micelle complex, are undoubtedly both involved in the pattern of results. These results should be confirmed by further studies. However, the results contribute further evidence to confirm the postulate that the surface-active agents do not cause damage to the mucous membranes, which was presented in an earlier paper (2). Sodium lauryl sulfate is known to be more hemolytic than is Tw 20; indeed, the latter compound is used in parenterals. The same relative activity has been shown with other types of biological membranes. It would appear, therefore, that had the surfactants damaged the mucous membranes of the rectal passage, the rate of absorption of the drugs in sodium lauryl sulfate solution should be shorter than the comparative solution containing Tw 20. The opposite results were obtained.

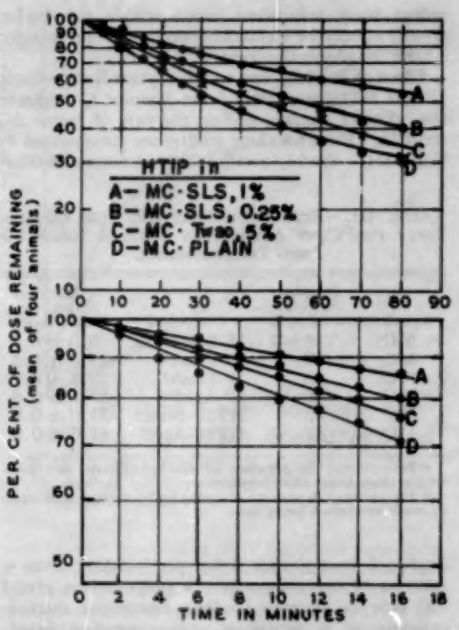

Fig. 2.—Rectal absorption curves for 2,4,6-triiodophenol in the methylcellulose vehicle and in the presence of several concentrations of two different surface-active agents. The data depict the marked retarding action of the surface-active agents.

Another interesting comparison can be made between the effects of identical concentration of surfactants on the absorption of free triiodophenol and its salt as reported previously (2). The absorption of both moieties is markedly retarded relative to systems containing no surface-active agents, as illustrated below in Table II.

TABLE II.—COMPARISON OF THE FIFTY PER CENT ABSORPTION TIMES OF TRIIODOPHENOL AND SODIUM TRIIODOPHENOLATE

Vehicle		50% Absorption Time ($t_{0.5}$)	
Type	Additivn	HTIP[a]	NaTIP[b]
MC	plain	35.0 ± 0.8	17.3 ± 1.5
MC	Tw 20-1%	49.9 ± 0.8	41.8 ± 0.8
MC	Tw 20-5%	28.5 ± 0.6	.....
MC	SLS-1%	73.3 ± 1	.....
CMC	SLS-1%	.....	59.3 ± 1.7
MC	SLS-5%	49.6 ± 0.9	.....
CMC	SLS-5%	.....	24.2 ± 0.3

[a] All HTIP vehicles were buffered with an acetate buffer to pH 4.6.
[b] All NaTIP vehicles were buffered with pyrophosphate to pH 8.6.

It should be noted that in the Tween systems, the time interval for 50% absorption of HTIP and NaTIP are in relatively close agreement. However, those listed for the SLS vehicles are significantly different. Anionic surfactants, such as SLS, are known to be much more affected by electrolytes than are nonionic surface-active agents, such as Tw 20. Apparently a change from a nonionic to an anionic surfactant, and a change from a mono-

valent to a polyvalent anion within the buffer greatly affects the surfactant micelle size and thereby affects the absorption rate.

Effect of Particle Size on Absorption Rate.—Both IF and HTIP were used in an attempt to evaluate the effect of particle size on the rate of rectal absorption. Data for these studies are summarized in Table III. Freshly precipitated iodoform and triiodophenol were prepared by precipitation from a solution of the compounds in polyethylene glycol 600 into an aqueous medium containing methylcellulose as a peptizing and suspending agent. This procedure produced a semicolloidal dispersion of the compounds which was [illegible] over several days' standing. The aged systems were precipitated in the absence of methylcellulose and aged eight to ten hours prior to the addition of the methylcellulose and the use of the suppository vehicle in absorption studies. The latter procedure allowed the compounds to crystallize into distinct, large crystal masses; therefore, a large difference in surface area resulted. This can be expected to produce a large difference in the rate of solution of the two different type solids, since solution rate is directly related to surface exposed to the solvent. Radioactive HTIP was present in the above vehicles in concentrations approximately one hundred times that of water saturation. No attempt was made to study HTIP in water-saturated solution. The radioactive HTIP in the vehicles studied gave an external counting rate of 400 to 500 counts per second. It was not experimentally feasible to synthesize TIP at a higher specific activity. A water-saturated system of HTIP would have resulted in an external initial counting rate of 4 to 5 counts per second. Such a counting rate would be difficult to evaluate since it is only a few counts above normal background activity.

TABLE III.—EFFECT OF PARTICLE SIZE ON THE FIFTY PER CENT ABSORPTION TIME OF IODOFORM AND TRIIODOPHENOL

Vehicle	Concentration (mg. %)	Compound	50% Absorption Time ($t_{1/2}$)
MC	8.	IF-Soln.	9.8 ± 0.6
MC	16.	IF-Fresh[a]	13.3 ± 0.5
MC	48.	IF-Fresh[a]	29.3 ± 0.7
MC	48.	IF-Aged[b]	37.1 ± 0.6
MC	32.	HTIP-Fresh[a]	35.0 ± 0.8
MC	32.	HTIP-Aged[b]	41.6 ± 0.8

[a] Precipitated in presence of methylcellulose and used within three hours after preparation.
[b] Precipitated in absence of methylcellulose and aged eight to ten hours before being used.

The IF concentrations range from saturation (8 mg. %) to approximately six times that of the water saturation value. A comparison of the different IF preparations shows that as the concentration of IF increases beyond water saturation, the rate of absorption decreases. This decrease in all probability is due to the solution of the suspended crystalline material. When the IF semicolloidal dispersions are allowed to age for twelve hours, larger crystals are formed and the absorption rate is further decreased. Similar results were obtained when these studies were repeated using freshly precipitated and aged samples of HTIP.

It is remarkable that HTIP is absorbed in such a relatively brief period of time. The experimental results show that one-half of the total amount of drug present, essentially all in the solid phase, dissolved in the vehicle and was absorbed during a thirty-five-minute period. The first paper of this series (1) presented evidence for the postulate that diffusion of the drug within the vehicle is the rate limiting step for the absorption process. It is possible that the rate of solution and, in turn, the rate of diffusion from the solid-liquid interface is the controlling factor for the pseudo-first order data obtained with these systems.

Absorption of Undissociated Compounds from Solid Vehicles.—Studies involving absorption of undissociated compounds from the rectum of the rat were also carried out using IF and HTIP in solid polyethylene glycol (SPEG), solid hard butter (SHB), and solid cocoa butter (SCB). The radioactivity was maintained at approximately the same level as used in the liquid systems. A summary of the resulting data is shown in Table IV and Figs. 3 and 4, with data for the liquid PEG vehicle included for comparative purposes.

Although their absolute solubilities were not determined, both IF and HTIP were observed to be much more soluble in PEG and oleaginous bases than in aqueous MC and CMC vehicles. In general, as much as one per cent HTIP was found to be soluble in SPEG, while less than half this amount appeared to dissolve in liquid cocoa butter and hard butter.

It is apparent in Figs. 3 and 4 that the normal shape of the absorption curve is affected during the first ten minutes by the melting characteristics of the vehicles. Experiments were conducted using supercooled, liquefied form of cocoa butter and hard butter. As may be expected, no significant differences in the rate of absorption, beyond the first ten minutes, were obtained.

TABLE IV.—FIFTY PER CENT ABSORPTION TIME FROM SOLID VEHICLES

Vehicle	Compound	50% Absorption Time ($t_{1/2}$)
LPEG	IF	23.2 ± 0.4
SPEG	IF	39.5 ± 1.4
SCB	IF	169.2 ± 9.1
LPEG	HTIP	43.8 ± 1.3
SPEG	HTIP	45.4 ± 0.9
SCB	HTIP	278.2 ± 9.6
SHB	HTIP	297.8 ± 12.8

Two different physical processes may account for the significant differences in time for 50% absorption of IF and HTIP from the liquid and solid vehicles. The latter group of vehicles soften or melt at body temperature, but the drug must partition from the oil to the water phase during the absorption process. This phenomenon presents a barrier to rapid absorption. The SPEG vehicle is a hypertonic system, and it must absorb water from the rectum for its dispersion (m. p., 52–55°) and eventual release of medicaments. HTIP is absorbed from SPEG at approximately the same rate as from LPEG, while there is a significant difference in time for 50% IF absorption from similar vehicles.

It is difficult to conclude whether the observed differences are due to the degree of complexing of the two drugs or due to the concentration of PEG in LPEG as compared to SPEG.

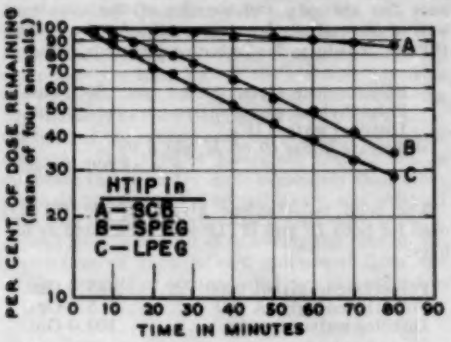

Fig. 3.—Rectal absorption curves for iodoform from a solid cocoa butter, solid polyethylene glycol and a liquid polyethylene glycol suppository vehicle.

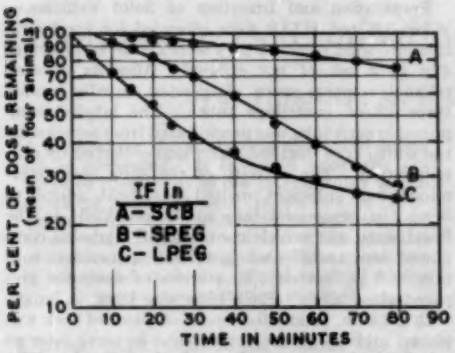

Fig. 4.—Rectal absorption curves for triiodophenol from solid oleaginous and polyethylene glycol suppository vehicles.

Head Count Studies.—A number of workers (5–9), during recent years, have endeavored to obtain kinetic data on drug absorption from the rectum by periodically sampling blood and detecting the amount of drug present. A brief study was made using an external counting method to determine the rate of accumulation of NaI-131, IF, and TIP in the area of the rat's head in an attempt to compare the rectal data obtained in the investigation with data that might be obtained by blood sampling. At the head, the level of radioactivity at any given moment should be related to the blood level of the absorbed compound.

The data obtained are represented graphically in Fig. 5. A comparison of curves A and B to curve C shows significant differences in accumulation rates of the three compounds within the head. One might arrive at two different sets of conclusions from comparison of the curves for IF and NaI-131: one set for the first twenty minutes of sampling, and a second set for the remainder of the experiment. If the results were used to interpret events taking place in the rectum, conclusions drawn

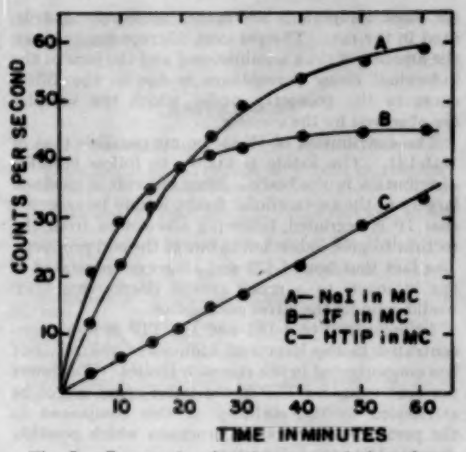

Fig. 5.—Curves showing the accumulation of rectally absorbed sodium iodide, iodoform and triiodophenol in the head of the rat.

could be very misleading since the rectal data show IF to be absorbed at a significantly faster rate than NaI-131. The head count data beyond the first twenty minutes appear to indicate that IF is absorbed from the rectum more slowly than NaI-131. The shape of the curves obtained in these studies may be related to the distribution of the absorbed compound in various tissues of the rat.

Distribution Studies.—At the end of the sixty-minute experimental head-count study, the animals were sacrificed and dissected in order to determine the distribution of the absorbed compounds in various organs and tissues. Table V summarizes the results for all three radio-tagged compounds.

TABLE V.—TISSUE DISTRIBUTION ONE HOUR AFTER RECTAL ABSORPTION OF RADIOACTIVE DOSE

Tissue	Gram Wet Weight[a]	Per Cent NaI-131[b]	Per Cent IF[b]	Per Cent TIP[b]
Blood	3.2 ± 0.5	3.0	2.8	2.5
Heart	0.6 ± 0.1	0.4	0.4	0.6
Spleen	0.7 ± 0.3	0.5	0.6	0.7
Lungs	1.7 ± 0.3	1.0	1.1	1.3
Thyroid	.....	0.4	0.5	0.3
Kidneys	1.4 ± 0.2	0.8	1.0	4.3
Urine	.....	0.7	0.9	1.1
Liver	7.0 ± 0.4	2.5	3.8	9.3
Stomach	2.9 ± 1.0	7.3	10.5	2.4
Small and Large Intestine	11.8 ± 2.3	6.4	5.3	7.9
Skin	27.8 ± 2.7	15.1	14.7	13.6
Rectal Portion Containing Dose	.....	23.5	21.8	28.9
Balance from Ground Carcass	117.0 ± 8.7	30.5	27.3	20.5
Total Dose Accounted For		92.1	90.7	93.4

[a] Pooled mean and standard deviation for three groups of four animals.
[b] Mean of four animals based on total dose administered.

With the exception of the kidneys, liver, ground carcass, and the rectal portion containing the dose,

all three compounds are rather uniformly distributed in the rat. The per cent discrepancy between the amount of drug administered and the sum of the individual tissue percentages is due to the differences in the geometry under which the samples are observed by the counter.

The distribution of IF in the rat parallels that of NaI-131. The iodide is known to follow chloride distribution in the body. Since chloride is confined largely to the extracellular fluids, it may be assumed that IF is degraded, following absorption from the rectum to give iodide ion as one of the end products. The fact that both I-131 and IF are concentrated in the stomach to a much greater degree than TIP would support the latter assumption.

As compared to I-131 and IF, TIP is more concentrated in the liver and kidneys of the rat, but less concentrated in the stomach tissues. The lower per cent value for TIP in the latter organ might be attributed to the stability of this compound in the presence of metabolic processes which possibly degrade IF to the iodide state.

During the NaI-131 distribution studies, a nonfasted rat was accidentally used; upon analysis, the stomach distribution value was found to be almost double that of the fasted animals. One might conclude, in view of this finding, that the blood levels are dependent on the degree of fasting of the test animal.

The amount of carrier in a vehicle influences the concentration of absorbed compound in the blood or various tissues; for example, Pearlman and coworkers (10) have found that when a tracer dose of NaI-131 containing 0.5 mg. inert potassium iodide is administered to rats, less than 2% is concentrated in the thyroid during a five-hour period. This value is in accord with our finding of 0.39%, found one hour after administering NaI-131 in the presence of approximately 0.33 mg. of inert sodium iodide.

EXPERIMENTAL

The radiological procedures and equipment, the pharmacological and counting procedures, the method of synthesis of HTIP and IF, the method of determining the radiochemical purity of HTIP, the assay of the radioactivity of the labeled compounds, and the physical characterization of the suppository vehicles have been described previously (1, 2).

Reagents.—The reagents used in this study which were not reviewed previously (1, 2) include: cocoa butter, U. S. P. grade, (Ghirardelli Co.) m. p. 34.5°; and hard butter, type M (Best Foods); a triglyceride substitute for cocoa butter possessing a softening point of 37.4°, saponification value 234, iodine value 2.9.

Formulas for Suppository Vehicles.—The basic methylcellulose (MC) vehicle used for both IF and HTIP is given below:

Polyethylene glycol 600	5.0 Gm.
Methylcellulose 4000 c. p. s.	1.5 Gm.
NaCl	*q. s.* to make isotonic
Buffer	*q. s. ad* 100.0 ml.

A 0.05 *M* phosphate buffer, pH 7.4, was used for IF; while a 0.05 *M* acetate buffer, pH 4.6, was used for HTIP. The surface-active agents were added to the above formulations in the required amounts (0.25 to 5%) and the vehicles were readjusted for tonicity and viscosity.

The isotonic liquid polyethylene glycol (LPEG) vehicle was designed to contain a large amount of purified polyethylene glycol 6000 in order to balance the viscosity and tonicity of the vehicle as well as possessing solvent properties for the IF and HTIP. The formulas for both compounds included:

Polyethylene glycol 6000	59.0 Gm.
Polyethylene glycol 600	5.0 Gm.
Distilled water (IF) *or* Acetate buffer (0.05 *M*, pH 5.9)	*q. s. ad* 100.0 ml.

The solid polyethylene glycol vehicle (SPEG) used for both IF and HTIP was formulated as follows:

Polyethylene glycol 6000	85.0 Gm.
Polyethylene glycol 600	5.0 Gm.
Distilled water to make	100.0 Gm.

Solid cocoa butter (SCB) vehicle and the solid hard butter (SHB) vehicle were used as received from the manufacturer.

Preparation and Insertion of Solid Vehicles.—When IF and HTIP were intended for incorporation into solid vehicles, they were dissolved individually in 3 ml. of hot ethanol. Aliquots of each ethanol solution were distributed equally among three 12-ml. centrifuge tubes. The labeled compound in each tube was precipitated from solution by the addition of distilled water and collected by centrifugation. The activity of the drug was determined and sufficient melted base was added to bring the compound into solution. While in the fused state, the vehicle containing the labeled compound was transferred in 0.2-ml. quantities, containing 5 μc. activity, to a series of specially prepared glass tubes. Each tube was 4 cm. in length with a 2-mm. inside diameter. A piece of cork was placed midway in the empty tube to serve later as a plunger for inserting the suppository.

When the solid preparations were administered, the glass tube containing the cork-suppository unit served as a distal rectal plug. Leakage of the vehicle to the outside was prevented by placing a layer of tape around the portion of the tube introduced into the anal opening. The cork remained in the orifice of the tube after delivery of the suppository to complete the seal.

Distribution Studies.—Each IF, TIP, and NaI-131 distribution study represents the mean of the data obtained from four rats, which were sacrificed one hour after the rectal administration of a 0.2-ml. dose of labeled substance in MC vehicles. The per cent of the dose in various tissues was determined by skinning each animal, dissecting its organs, and grinding up the remains in a meat grinder. The skin and ground portions were put in separate round paper containers while the organs were placed on small round tin planchets. In order to correct the geometry and radioactive decay, an aliquot portion of the dose was run on the same shelf, in a similar planchet, and at the same time as the tissues. From the aliquot portion (1 ml.) of the diluted dose (0.2 ml. *q. s. ad* 100 ml.) the total number of counts per second for the dose administered was calculated. The activity of the labeled compound in each organ

was then expressed as a percentage of the calculated value. A second scintillation counter was used for this procedure.

Head Count Studies.—Each IF, TIP, and NaI-131 head count study represents the mean of the data obtained from four rats. Each rat was prepared in the same manner as described for the rectal studies using MC vehicles with the following exception—the hind legs of the rat were taped to the plexiglass with the upper extremities extending over the edge of the plexiglass support so as to allow the head of the rat to cover the one-inch collimator opening. This type of placement allowed one to duplicate the geometry more accurately than other methods attempted. The collimator was shielded with lead bricks so that initial counts were at background level. Instead of following the rate of disappearance of each labeled compound from the rectum, its rate of accumulation was determined in the head of the animal at five-minute intervals for the first hour.

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Colorimetric Determination of Thymol in Thyme Oil*

By L. FIBRANZ, M. I. BLAKE, and C. E. MILLER

A method for the analysis of thymol in thyme oil is presented. It is based upon the absorptive properties of the yellow color which develops when thymol is treated with *p*-dimethylaminobenzaldehyde in sulfuric acid as solvent. Both the red and white varieties of thyme oil are investigated. The procedure is compared with the official method and a nonaqueous titration method.

THYME OIL, typical of the volatile oils which contain predominantly phenolic constituents, is assayed by treatment with an alkali hydroxide (1). According to Guenther (2) this technique was first applied by Gildemeister for the determination of phenols in thyme oil. It is now a general method for the analysis of phenols in volatile oils.

Guenther points out several factors which may lead to erroneous results. Any alkali-soluble or water-soluble constituents or adulterants will dissolve in the aqueous phase and will be calculated as phenols. An aqueous solution of alkali phenoxide is a better solvent for the nonphenolic portion of an oil than is the alkali solution. The use of large sample volumes (10 ml.), the difficulty of reading the meniscus accurately, and the lengthy reaction period (overnight for thyme oil) are additional drawbacks. Experimental work in this laboratory indicates that good precision is not attainable with this assay procedure.

Menthol in peppermint oil has recently (3) been accurately determined by measuring the absorptive properties of the red color which develops when menthol is treated with *p*-dimethylaminobenzaldehyde. The present report describes a similar reaction for thymol. The method is applied to the quantitative determination of thymol in thyme oil and is compared with the official procedure and a nonaqueous titration method.

EXPERIMENTAL

Color Reagent.—The color reagent was prepared by dissolving 125 mg. of *p*-dimethylaminobenzaldehyde, reagent grade, in 100 ml. of concentrated sulfuric acid, reagent grade. Fresh reagent was prepared daily.

Standard Curve for Thymol.—A standard thymol solution was prepared by weighing 55.0 mg. of N. F. thymol into a 50-ml. volumetric flask. Chloroform, U. S. P. grade, was added to the mark. Exactly 1.0 ml. of the standard solution was transferred by pipet into a 25-ml. volumetric flask and the flask was diluted to the mark with chloroform. Each milliliter of the second dilution contained 44.0 μg. of thymol. A standard curve was prepared from aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 ml. of the second dilution. This represented a concentration range for thymol of 8.8 to 44.0 μg. The aliquots were transferred to 50-ml., glass-stoppered graduates. Exactly 5.0 ml. of color reagent was added from a buret to each cylinder. The cylinders were stoppered and shaken briskly for about ten seconds

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to hasten the color reaction. A bright yellow color developed immediately in each cylinder varying in intensity with concentration of thymol. The colored solutions were transferred to colorimeter tubes and readings were taken with the Klett-Summerson photoelectric colorimeter using the blue filter No. 42 (400–465 mμ). An average of three readings was recorded for each of the solutions. One milliliter of chloroform and 5.0 ml. of color reagent were treated in the same manner as the thymol solutions and served as the blank. Scale readings were directly proportional to the concentration of thymol and the absorbance of the colored solution. A standard curve for thymol was constructed by plotting scale readings *vs.* thymol concentrations on ordinary graph paper. The range of concentrations used in this study obeyed Beer's law.

Color Development.—In a preliminary study an attempt was made to construct a standard curve using *p*-dimethylaminobenzaldehyde T. S., U. S. P. (4), as the color reagent. This solution contains 35 ml. of water, 65 ml. of sulfuric acid, and 125 mg. of *p*-dimethylaminobenzaldehyde in each 100 ml. Although excellent results were reported for menthol (3), reproducible results were not obtained with thymol in the present study. Standardizing the conditions for color production was next considered. Each aliquot of standard thymol solution was treated in the same manner with color reagent and all were shaken simultaneously by means of an International Bottle Shaker for a specific length of time. The color intensities were noted after a definite time period. Repeated runs were conducted varying the shaking time from five minutes to twenty minutes, and the overall time period for color development was varied from ten minutes to two hours. Satisfactory results were not obtained. The composition of the color reagent was then investigated. In another series of tests the relative concentration of acid and water was varied. When concentrated sulfuric acid was used as the solvent for the color reagent, constant and reproducible results were obtained. Uniformity of shaking and the time factor were of little importance. The same concentrations used for establishing the calibration curve were employed for this study. After the color reagent was added to the aliquots of standard thymol solution, the cylinders were shaken by hand for about ten seconds. The color intensities were then read immediately, after a five-minute waiting period; and subsequently, after fifteen-minute time intervals, for a total time period of two hours. Scale readings after the first series varied less than 2% from the original readings.

Analysis of Thyme Oil.—Oil of thyme was placed into a two-dram dropper bottle and the system was weighed. The white (commercial grade) and red (N. F. VII) varieties of thyme oil were used in this study. Approximately 100 mg. of oil (3 to 4 drops), accurately weighed, was removed from the dropper bottle and placed into a 50-ml. volumetric flask. The flask was filled to the mark with chloroform. One milliliter of this solution was transferred by pipet into a 25-ml. volumetric flask and the second flask was subsequently filled to the mark with chloroform. The concentration of thyme oil in the second flask was approximately 80.0 μg./ml. or about twice the concentration of the standard thymol solution used to prepare the standard curve. Exactly 1.0 ml. of the second dilution was transferred by pipet into a 50-ml. glass stoppered cylinder and was treated with color reagent as described previously for the preparation of the standard curve. Three additional 1.0-ml. aliquots were treated similarly. The concentration of thymol was determined by applying the photometer reading to the standard curve. The average for the four aliquots was calculated. The precision was better than 1 part per 1,000. Four separate analyses were run on both the red and white thyme oils. The results are reported in Table I. The second column of the table indicates the concentration of thymol in each ml. of the second dilution. Column three shows the thymol concentration as determined from the standard curve and represents the average of four samples run concurrently. The phenol percentage of the oil, determined as thymol, is listed in the fourth column.

TABLE I.—COLORIMETRIC DETERMINATION OF THYMOL IN THYME OIL

Assay No.	Thyme Oil Concn.[a] (μg./ml.)	Thymol Concn.[a] (μg./ml.)	Phenol,[b] %
Red Variety			
1	82.4	23.7	28.76
2	81.7	23.6	28.89
3	82.3	23.3	28.31
4	80.1	23.2	28.96
		Av.	28.73
		Av. Dev.	0.21
White Variety			
1	77.3	16.6	21.47
2	77.3	16.8	21.73
3	79.6	17.0	21.36
4	80.3	17.6	21.92
		Av.	21.62
		Av. Dev.	0.20

[a] Concentration per ml. of second dilution.
[b] Determined as thymol.

Interfering Substances.—Of the substituents normally occurring in thyme oil as recorded by Guenther (5), linalool, α-pinene, *p*-cymene, geraniol, and borneol, all in high concentrations, produce color with the reagent. For each constituent a standard solution was prepared as described earlier for thymol, except that the concentrations were about one-half that of the thymol standard solution. These dilutions represented concentrations of the constituents many times those in which they normally appear in thyme oil. In each instance the intensity of color as recorded by the photometer was not significant. It is conceivable, however, that if any one of these constituents were present in the oil in unusually large amounts, it would be possible for it to interfere with the analysis.

Official Assay of Thyme Oil.—Both varieties of thyme oil were assayed as directed in N. F. X (1). The results are shown in Table II.

Nonaqueous Titration of Thyme Oil for Phenol Content[1]—Preparation of 0.1 N Sodium Methoxide.—To 100 ml. of absolute methanol, reagent grade,

[1] More complete details of this phase of the work will be reported by one of the authors (M. I. B.) in a subsequent publication dealing with the analysis of phenol-containing volatile oils.

approximately 5 Gm. of clean sodium was added slowly while the mixture was immersed in an ice bath. After all the sodium dissolved an additional 150 ml. of methanol was added, followed by 1,500 ml. of dry benzene. The solution was stored in the reservoir of an automatic buret protected from moisture and carbon dioxide in the atmosphere. The normality of the solution was determined by standardization against reagent grade benzoic acid.

TABLE II.—OFFICIAL ASSAY OF THYME OIL

Assay No.	Red Thyme Oil, % Phenols	White Thyme Oil, % Phenols
1	28.5	21.4
2	29.5	21.5
3	29.0	22.0
4	28.9	22.7
Av.	29.0	21.9
Av. Dev.	0.3	0.4

Assay Procedure.—Approximately 600 mg. of thyme oil, accurately weighed, was transferred from the dropper bottle into a 150-ml. beaker. About 30 ml. of N,N-dimethylformamide was added. The solution, magnetically stirred, was titrated with 0.1 *N* sodium methoxide solution using a Fisher titrimeter equipped with a sleeve-type calomel electrode and a platinum electrode. The end point was determined by plotting millivolts *vs.* milliliters of titrant. The data are shown in Table III. The phenol content, determined as thymol, was calculated from the expression:

$$(\text{ml.} \times N \times 150.21 \times 100)/\text{sample weight} = \% \text{ phenols (thymol)}$$

where ml. = milliliters of sodium methoxide solution, N = normality of sodium methoxide solution, 150.21 = mEq. weight of thymol, and sample weight = weight of sample in mg.

TABLE III.—NONAQUEOUS TITRATION OF THYME OIL

Assay No.	Red Thyme Oil, % Phenols	White Thyme Oil, % Phenols
1	28.65	21.25
2	29.01	21.81
3	29.11	21.93
4	28.58	21.55
Av.	28.84	21.64
Av. Dev.	0.22	0.24

RESULTS AND DISCUSSION

The many shortcomings of the official assay procedure for determining phenols in volatile oils prompted the investigation for an improved method of analysis. The procedure described in this report is based upon a method presented earlier (3) for the estimation of free menthol in peppermint oil. It was necessary, however, to modify the color reagent since *p*-dimethylaminobenzaldehyde T. S., U. S. P., did not give constant and reproducible results. The thymol concentrations employed in this study followed Beer's law when the color reagent was prepared with undiluted sulfuric acid as solvent. Both the red and white varieties of thyme oil were studied. The results are reported in Table I. For the white thyme oil an average thymol content of 21.62 ± 0.20 is shown and for the red variety the thymol content is 28.73 ± 0.21. According to N. F. X (1), thyme oil must contain not less than 40% phenols to meet official specifications. However, the thyme oil used in this investigation was labeled N. F. VII. According to that edition of the N. F. (6), the phenol content of thyme oil must be not less than 20%. The white thyme oil used in this study was a commercial variety and corresponded to N. F. VII standards.

The colorimetric method described in this paper has a number of advantages. Once the standard curve has been established, the routine analysis of thyme oil can be effected in less than an hour, whereas the official assay requires an overnight waiting period. Sample weights of less than one gram are needed in place of the 10-ml. for the official method. Results are thus expressed in terms of per cent weight in weight and not volume in volume. The former is the usual manner for expressing the percentage strength of constituents. Greater accuracy and precision are attainable. Inaccuracies which result from the difficulty of reading the meniscus and from the solubility of nonphenolic alkali-soluble impurities present no problem.

The possibility of interfering substituents cannot be overlooked. Several of the components of thyme oil produce color with the reagent. The concentration of these constituents is normally so small in comparison to the thymol content that interference is negligible. To substantiate this fact the proposed assay procedure was compared with the official assay. Comparable results were obtained (Tables I and II). Further supportive evidence was obtained by comparison with a nonaqueous titration method. Similar results are reported (Table III). This last method will be treated more fully in a subsequent publication.

SUMMARY AND CONCLUSIONS

1. A method for the analysis of thymol in thyme oil is presented. It is based upon the color reaction between thymol and *p*-dimethylaminobenaldehyde. Concentrations of thymol ranging from 8.8 to 44.0 μg./ml. obey Beer's law.

2. Quantitative results are presented for the analysis of red and white varieties of thyme oil. The possible interference of other constituents is considered.

3. The method is compared with the official assay procedure and with a nonaqueous titration method.

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Sulfaethylthiadiazole III.*

Kinetics of Absorption, Distribution, and Excretion

By JOSEPH V. SWINTOSKY, ELWOOD L. FOLTZ,† AMEDEO BONDI, JR.,‡ and MANFORD J. ROBINSON

Experimental data are presented which describe blood concentrations during the early absorptive and post-absorptive periods following oral sulfaethylthiadiazole administration to humans. Data from urinary collections over forty-eight hours are also given. These and previously reported data are employed to illustrate and interpret the kinetics of sulfaethylthiadiazole absorption, distribution, and excretion. The pharmaceutical implications with regard to design and evaluation of data from dosage forms, and their regimens, are discussed.

PREVIOUS PAPERS (1, 2, 3) have reported the approximate first-order elimination rate of sulfaethylthiadiazole (SETD) as determined from human blood concentration and urinary excretion studies following intravenous and oral administration. Laboratory and clinical efficacy of SETD have also been reported (4, 5). This paper describes blood concentrations, observed during the early absorptive and post-absorptive phases; and urinary excretion data following oral SETD administration. These additional experimental data are correlated with data previously reported. The kinetics of SETD absorption, distribution, and excretion following oral and intravenous doses are interpreted in terms of these data, and some pharmaceutical implications related to problems of dosage form design are discussed.

EXPERIMENTAL

Eight healthy ambulatory adult human subjects were employed. Doses of SETD ranging from 1 to 4 Gm. were administered orally in 0.5-Gm. hard gelatin capsules just prior to breakfast, after control blood samples had been taken. Blood collections for sulfonamide concentration were made by venipuncture at 20, 30, 45, 65, 90, 120, 150, 180, and 240 minutes following administration.

In another study employing four subjects at the same oral doses, blood and urine collections were made. Following breakfast, the subjects were permitted to ingest foods and fluids as desired but were urged to consume a minimum of 300 cc. of water at each urine collection time. Collections of total urinary output were made at 0, 3, 6, 9, 12, 15, 24, and 48 hours. Blood concentration data were obtained from 0–10 hours and were interpreted previously (1).

Analytical Methods.—Concentrations of free as well as free plus conjugated (total) SETD in the blood and urine were determined by a modification of the spectrophotometric macromethod of Frisk (6), as reported previously (1, 2).

RESULTS AND DISCUSSION

Blood Concentrations During First Four Hours.—The data for the eight subjects tested are given in Table I, and indicate rapid absorption. In almost all instances measurable blood concentrations were observed within twenty minutes; and with one exception, all subjects exhibited concentrations of free SETD equal to or in excess of 5 mg. % in sixty-five minutes. Peak blood concentrations generally occurred within one to three hours.

Urine Collection Studies.—The experimental urinary excretion data for four human subjects receiving SETD are presented in Table II. The close correspondence of free and total SETD excreted in the various time intervals corroborates results from intravenous administration studies that biotransformation is of a very low order (2). About 79–90% of the administered dose is excreted via the urinary tract in forty-eight hours. This contrasts with 83–100% excretion in forty-eight hours for subjects receiving 0.5–2.0 Gm. intravenous doses (2). After oral doses, an average of 85% of the drug was excreted in forty-eight hours; after intravenous doses, 91% was excreted in the same period. For these subjects one may assume for purpose of calculations that 93% of the total oral dose was absorbed and eventually excreted in the urine.

When the average total drug excreted during the three- to fifteen-hour interval is plotted by a differential method illustrated previously (2, 7), the biologic half-lives are of magnitudes observed for intravenous doses determined similarly. These plots are illustrated in Fig. 1. Biologic half-lives vary between six to eight hours. These and previous blood and urine concentration studies with SETD indicate that the biologic half-lives usually are within five to thirteen hours for the adult human subjects studied.

Distribution Volume.—It has been shown (2) for SETD that the apparent volume of distribution, V_b, determined from blood concentration data, may be estimated from the equation

$$V_b = W/C \qquad \text{(Eq. 1)}$$

where, W = mg. of total drug in the body at time t

* Received August 24, 1957, from the Smith, Kline & French Laboratories, Philadelphia 1, Pa.

† Present address: Section of Infectious Diseases, Department of Medicine and the William Pepper Laboratory of Clinical Medicine, University of Pennsylvania School of Medicine, Philadelphia.

‡ Present address: Division of Microbiology, Hahnemann Medical School and Hospital, Philadelphia 1, Pa.

TABLE I.—BLOOD CONCENTRATION DATA DURING THE ABSORPTIVE AND EARLY POST-ABSORPTIVE PHASES FOR ADULT MALE SUBJECTS[a] RECEIVING SETD AT SEVERAL DOSAGE LEVELS

Minutes	Dose (Gm.)	Subjects and Their Free (F) and Total (T) SETD Blood Concentration in mg. %: Bd F	Bd T	Bs[b] F	Bs[b] T	Hn F	Hn T	Ro[b] F	Ro[b] T	Mt F	Mt T	Ly F	Ly T	Bi F	Bi T	Sa F	Sa T
20	1.0	0.5	0.7	2.8	3.1	0.5	0.7	0.0	0.0	1.4	1.4	1.0	1.0	1.1	1.1	1.1	1.1
30		1.4	1.4	3.2	3.4	2.5	2.7	1.2	1.3	4.4	4.4	1.5	1.5	2.0	2.0	1.2	1.3
45		4.3	4.4	3.8	4.2	4.6	4.8	2.1	3.6	5.7	6.1	5.1	5.1	5.3	5.3	2.0	2.1
65		8.5	9.2	5.0	5.6	8.8	8.5	3.2	3.6	7.1	7.5	6.3	7.0	5.4	5.7	5.8	6.1
90		7.6	8.4	5.0	5.4	7.0	7.2	5.3	5.5	6.1	6.3	8.8	9.4	6.1	6.5	7.1	7.5
120		6.9	7.3	4.8	5.2	6.4	6.9	4.8	5.6	5.4	5.5	9.0	9.8	5.7	6.3	7.8	7.9
150		6.4	6.6	4.2	4.8	5.1	6.1	4.5	5.2	5.3	5.4	8.4	8.5	6.3	6.5	7.9	8.1
180		5.7	5.8	3.9	4.3	5.1	5.5	4.2	4.8	5.3	5.4	7.9	8.4	8.1	6.3	7.1	7.1
240		5.1	5.3	3.5	3.9	4.4	5.0	3.8	4.1	5.1	5.3	7.1	7.5	6.3	6.5	7.0	7.0
20	2.0	0.4	0.5	2.5	2.6	0.0	0.0	0.0	0.0								
30		4.0	4.1	7.2	7.9	0.4	0.3	0.0	0.0								
45		8.0	8.2	9.1	9.6	1.6	1.6	0.0	0.0								
65		11.6	11.8	9.8	10.4	10.2	10.2	5.0	5.0								
90		11.8	12.5	9.4	9.2	12.4	13.0	9.4	9.6								
120		10.2	11.0	9.0	9.1	11.2	11.6	10.2	10.2								
150		9.3	9.8	8.0	8.4	10.2	10.6	9.6	9.8								
180		9.0	9.7	7.6	7.6	9.4	9.6	8.8	9.3								
240		8.0	8.0	6.6	6.8	7.6	8.1	8.2	8.6								
20	3.0	...	...	...	...	...	...	...	...	0.9	1.0	...	...	0.8	0.9	1.4	1.5
30		...	...	...	...	...	...	...	...	1.4	1.5	...	...	1.0	1.1	5.3	5.4
45		...	...	...	...	...	...	...	...	2.1	3.0	...	...	6.7	7.1	7.0	8.1
65		...	...	...	...	...	...	...	...	7.7	7.9	...	...	9.2	10.8	8.5	9.2
90		...	...	...	...	...	...	...	...	7.9	8.0	...	...	12.5	13.0	9.2	9.3
120		...	...	...	...	...	...	...	...	8.5	9.7	...	...	14.0	12.3	11.3	11.8
150		...	...	...	...	...	...	...	...	13.1	13.1	...	...	15.2	15.6	12.5	13.1
180		...	...	...	...	...	...	...	...	12.5	13.0	...	...	15.8	16.4	13.1	14.7
240		...	...	...	...	...	...	...	...	12.0	12.5	...	...	11.8	13.1	13.1	13.0
20	4.0	2.7	2.8	9.1	9.7	2.0	2.6	0.0	0.0								
30		7.7	7.6	10.8	12.6	7.4	7.5	0.4	1.0								
45		10.9	10.6	12.0	12.7	19.7	11.2	2.2	3.4								
65		14.0	14.5	13.4	14.0	12.0	12.1	6.9	7.8								
90		18.2	18.2	13.9	14.6	17.5	19.2	11.8	12.6								
120		20.0	20.2	14.1	14.2	17.2	17.2	13.4	14.4								
150		18.6	19.8	13.5	13.5	15.1	15.6	13.5	14.4								
180		18.2	18.4	12.8	13.4	14.6	14.6	11.4	12.8								
240		16.4	15.9	11.5	12.0	13.2	13.8	10.6	11.8								

[a] Subject ages and weights were as follows:

Subject	Age (yr.)	Weight (Kg.)	Subject	Age (yr.)	Weight (Kg.)
Bd	23	75	Mt	30	70
Bs	38	114	Ly	26	73
Hn	22	84	Bi	44	86
Ro	22	91	Sa	27	71

[b] Blood concentration data at longer time intervals for oral and intravenous doses are reported for these subjects in previous papers (1, 2).

(and theoretically exclusive of any drug in the gastrointestinal tract and bladder); and C = mg./cc. of total drug in the blood at time t, after establishment of diffusion equilibrium.

In light of this finding from intravenous studies on these same four subjects, it seemed desirable to determine if V_b had a similar value following oral dosage. As a corollary, it was of interest to establish further whether blood and urine data following oral doses would be correlated semi-quantitatively with results observed following intravenous doses. The pertinent experimental data and calculations are presented in Table III. In all calculations it was assumed that only 93% of the oral doses was absorbed. The average per cent distribution volume, V_b %, is observed to be about 20%, contrasting with 18% from intravenous studies. The correspondence is fair; however, the result from intravenous studies involves fewer assumptions and is therefore more reliable. Figure 2 is a plot of W *vs.* C from the data of Table III. Data for three subjects plot fairly satisfactorily as straight lines approaching intersection with the origin. For the fourth subject the data did not show this consistency. The fair similarity between these plots and those observed following intravenous doses (2) supports the postulation that approximately a linear relationship exists between total SETD blood concentration and drug in the body. This relationship exists for blood concentrations up to at least 12–15 mg. % for these subjects.

Some Interpretations Applied to Experimental Data of a Single Subject.—The data of Tables I and II lend themselves to interesting theoretical interpretations and descriptive illustrations. Subject Bs has been studied intensively (1, 2) and has shown an SETD disappearance rate *via* blood and urine concentration studies quite typical of the average subject. It is convenient, therefore, to use data for this subject, following a 2-Gm. dose, to illustrate some of the interpretations pertaining to absorption and excretion that apply similarly to all the subjects.

TABLE II.—URINARY EXCRETION DATA FOR HUMAN SUBJECTS RECEIVING ORAL SETD[a]

		Urinary Volumes In cc. and Drug Excreted in Mg. for Intervals Indicated											
		1.0 Gm. Dose			2.0 Gm. Dose			3.0 Gm. Dose			6.0 Gm. Dose		
Subject	Collection Time Interval (hr.)	Urine vol. (cc.)	Free mg.	Tot.[b] mg.	Urine vol. (cc.)	Free mg.	Tot. mg.	Urine vol. (cc.)	Free mg.	Tot. mg.	Urine vol. (cc.)	Free mg.	Tot. mg.
Ro	0–3	160	141	160	250	340	369	680	571	578	135	713	770
Ro	3–6	190	122	125	175	287	312	200	480	540	163	737	782
Ro	6–9	152	94	100	175	266	294	213	320	330	166	478	523
Ro	9–12	160	67	77	260	166	174	865	277	294	133	261	279
Ro	12–15	235	80	96	165	109	112	330	165	188	96	175	187
Ro	15–24	335	77	87	495	193	193	710	284	298	467	411	453
Ro	24–48	1240	130	143	1760	220	229	1790	269	295	1470	338	397
Bs	0–3	300	174	180	115	340	385	385	647	712	118	699	773
Bs	3–6	1050	147	179	400	288	320	915	458	458	290	696	725
Bs	6–9	225	81	97	335	228	251	312	362	406	235	475	576
Bs	9–12	340	68	75	305	153	171	184	184	184	110	198	239
Bs	12–15	165	73	79	125	129	138	178	142	196	122	203	232
Bs	15–24	780	129	156	1020	255	291	750	270	315	625	450	463
Bs	24–48	1365	109	154	1300	189	233	1225	257	276	1443	375	375
Cw	0–3	72	109	119	190	365	376	220	757	836	435	726	740
Cw	3–6	84	148	166	660	325	351	455	505	569	570	536	604
Cw	6–9	125	150	153	100	182	190	120	221	252	150	420	435
Cw	9–12	130	81	85	95	114	126	78	225	250	248	441	477
Cw	12–15	510	92	92	160	186	192	125	175	194	340	187	204
Cw	15–24	2015	111	141	715	164	168	540	286	313	445	343	513
Cw	24–48	2580	129	142	1155	225	231	1350	277	297	2795	503	601
Ds	0–3	80	131	132	95	327	447	120	298	330	130	551	579
Ds	3–6	73	99	113	120	322	366	168	497	546	170	571	617
Ds	6–9	115	129	129	300	180	198	65	211	241	425	544	638
Ds	9–12	74	62	69	73	93	112	105	313	368	425	323	361
Ds	12–15	80	51	56	85	133	136	125	214	225	325	133	153
Ds	15–24	590	130	148	245	218	206	820	369	410	485	340	393
Ds	24–48	1035	176	186	1065	197	245	1220	329	378	1305	653	718

[a] Data for the same subjects receiving intravenous SETD are reported in a previous paper (2).
[b] Total drug represents free and conjugated drug expressed in mg. equivalents of SETD.

TABLE III.—DATA CALCULATED FROM BLOOD CONCENTRATION AND URINARY EXCRETION STUDIES ON FOUR HUMAN SUBJECTS RECEIVING ORAL SETD

Subject	Height (in.)	Wgt. (Kg.)	Age (yr.)	Dose (Gm.)	Total SETD blood concentration at 3 hrs. (mg. %)	Total drug[a] in the body at 3 hrs. (mg.)	V_b (cc.) From 3-hr. data	Total SETD blood concentration at 6 hrs. (mg. %)	Total drug[a] in the body at 6 hrs. (mg.)	V_b (cc.) From 6-hr. data	V_b (cc.) Average from 3- and 6-hr. data	V_b % (V_b/V_s)
Ro	78	91	22	1.0	4.3	770	19,700	3.5	645	20,300		
				2.0	7.8	1491	21,000	6.3	1179	20,600		
				3.0	11.3	2212	21,500	8.5	1672	21,600		
				4.0	13.8	...	...	11.8	...	...		
				...							21,000	23
Bs	72	114	38	1.0	4.1	750	16,100	2.9	571	17,300		
				2.0	8.0	1475	16,200	5.5	1155	18,400		
				3.0	9.1	2078	20,100	6.6	1620	21,500		
				4.0	12.2	2947	21,200	9.2	2222	20,200		
				...							19,000	17
Cw	74	91	22	1.0	6.1	811	14,600	4.6	645	15,400		
				2.0	9.0	1484	18,100	7.3	1133	17,100		
				3.0	12.3	1954	17,500	8.8	1385	17,300		
				4.0	16.8	2980	19,500	13.6	2376	19,200		
				...							17,000	19
Ds	71	81	25	1.0	7.4	798	13,300	6.1	685	13,900		
				2.0	11.0	1413	15,900	9.0	1047	14,400		
				3.0	...	...	...	...	...	...		
				4.0	15.3	3141	25,400	14.3	2624	22,700		
				...							18,000	22

[a] Knowing the doses administered and assuming 93% absorption, the amounts of total SETD remaining in the body are determined by subtracting the cumulative amounts found in the urine at three and six hours respectively (Table II) from the amounts absorbed.

The rate of SETD blood concentration change following an oral dose depends primarily on rate of absorption, sequestering and storage in specific tissues, rate and degree of biotransformation, rate of distribution between blood and other accessible body tissues, and rate of excretion. Previous data (1–3) have indicated that biotransformation, storage, and sequestering are minimal, and distribution proceeds at a rate similar to the absorption rate. Thus it is reasonable to assume that diffusion equilib-

rium is very nearly maintained during the absorption process, and that absorption and urinary excretion rates constitute the principal limiting rates which govern blood, body and urine content of a dose as a function of time. With these assumptions, and knowing that total SETD disappearance from the blood or body at therapeutic concentrations approximates a simple decreasing exponential rate after termination of absorption, equations similar in principle but different in form from those of Dominguez, *et al.* (8), and Teorell (9) may be applied to the experimental data. Use of these equations in conjunction with the experimental data for SETD permits a delineation of the interrelationships of absorption, distribution, body drug content, blood concentration, and urinary excretion as related to time.

Thus for the total SETD, let W be the amount which is present in body tissues at a given time. Some additional drug may or may not be in the gastrointestinal and urinary tracts. The rate at which W changes is equal to the difference between the rate of absorption dA/dt, and the rate of excretion dE/dt, i.e.

$$(dW/dt) = (dA/dt) - (dE/dt) \qquad \text{(Eq. 2)}$$

When $dA/dt = dE/dt$, W becomes constant and dW/dt therefore is zero, a consideration of practical interest in the design of oral sustained release dosage forms. One might predict from theory that such a dosage form could be designed to release a therapeutic dose for immediate absorption, and an amount each hour thereafter corresponding to the amount excreted per hour. The sustained release dose would be the product of total drug in the tissues corresponding to the desired therapeutic level, the specific velocity constant for drug elimination, and the number of hours such sustained absorption was desired (1–3, 10). In practice it is observed that other factors have a practical bearing on these calculations of dosage form design. These

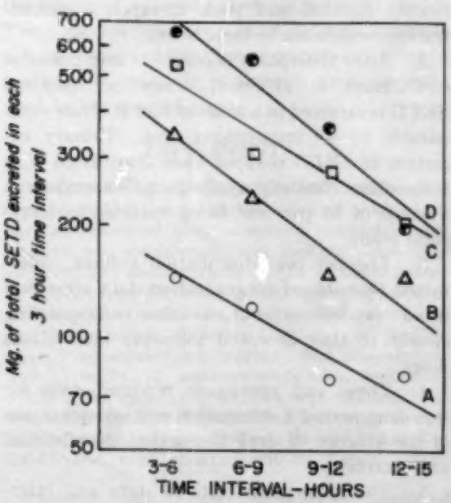

Fig. 1.—Plots of the average total SETD urinary excretions for 4 human subjects following oral doses of (A) 1.0, (B) 2.0, (C) 3.0, and (D) 4.0 Gm.

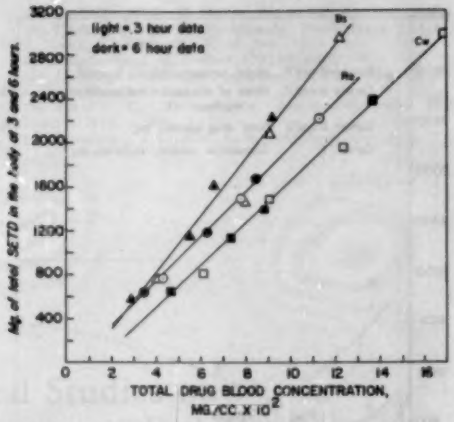

Fig. 2.—A plot of total SETD in the body *vs.* blood concentrations for subjects Bs, Ro, and Cw.

vary from one drug to another and for some drugs practically no oral sustained absorption can be effected by current technology.

Since V_b is assumed to be constant, and from Eq. 1, $W = V_bC$, one may substitute and transpose equivalent terms in Eq. 2 to obtain:

$$(dA/dt) = (V_bdC) + (dE/dt) \qquad \text{(Eq. 3)}$$

The rate of total SETD urinary excretion has been shown to be approximately proportional to SETD concentration in the blood (2) and may be written:

$$dE/dt = k_bV_bC \qquad \text{(Eq. 4)}$$

where k_b is the specific velocity constant for drug elimination determined from blood concentration data. Substituting this equivalent value of dE/dt in Eq. 3

$$(dA/dt) = (V_bdC/dt) + (k_bV_bC) \qquad \text{(Eq. 5)}$$

After absorption ceases, the total SETD concentration in the blood falls, as previously reported (2), approximately according to the equation:

$$\log C = -(k_bt/2.303) + \log Co \qquad \text{(Eq. 6)}$$

The values of k_b, V_b, and $V_b\%$ for subject Bs have been shown to be approximately 0.09, 20,000 cc., and 18% respectively (1, 2). Assuming 93% absorption of the oral doses, and employing the equations and constants enumerated above, the data in Tables I and II and those previously reported (1, 2) for subject Bs at a 2-Gm. dose, may be represented as shown in Fig. 3.

Curve *A* delineates the experimental blood concentration through ten hours (1) following oral administration. The remainder of the curve is extrapolated by use of Eq. 6. Curve *B* describes absorption rate of SETD from the data in Table I as interpreted with Eq. 5. It is evident that absorption is very rapid. The peak rate lies between 3000 and 4000 mg./hour, and is approximately 15–20 times faster than the peak excretion rate. The curve indicates that for this subject virtually all the absorbable drug is taken into the body within one to two hours. The area under the curve is a

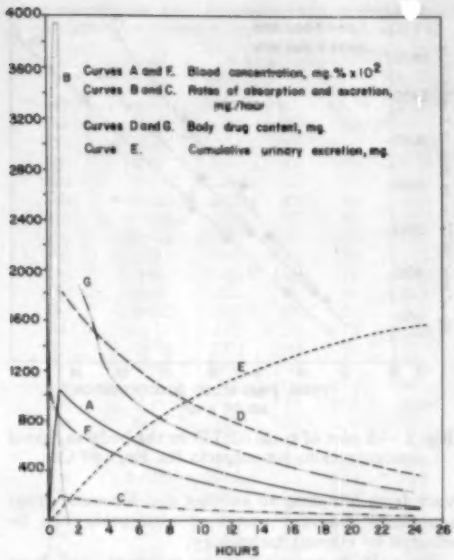
Curves A and F. Blood concentration, mg.% x 10²
Curves B and C. Rates of absorption and excretion, mg./hour
Curves D and G. Body drug content, mg.
Curve E. Cumulative urinary excretion, mg.
HOURS

Fig. 3.—Total SETD data for subject Bs showing (A) blood concentration, (B) absorption rate, (C) excretion rate, (D) body content, (E) cumulative urinary collection curves after 2-Gm. oral dose, (F) blood concentration, and (G) body content curves after 2-Gm. intravenous dose.

measure of total drug absorbed (8); however, this quantity is determined indirectly with greater precision from urinary collection data.

Curve *C* describes SETD disappearance rate from the blood for an oral dose, calculated according to Eq. 4. Essentially the same curve is obtainable in from three–twenty-four hours using the urinary excretion data of Table II. The area under curve *C* is a direct measure of the total drug excreted in twenty-four hours; however, this quantity too is determined with greater precision from urinary collection data.

Curves *D* and *E* describe body content of the drug and drug collected cumulatively in the urine, respectively, with regard to time, from data of Table II. Curve *F* illustrates the experimental blood concentrations observed through eight hours following intravenous administration (2); the remainder of the curve was obtained by extrapolation employing Eq. 6. Curve *G* illustrates the experimental urinary excretion data corresponding to the blood concentration data of curve *F*. The urinary excretion rate is very rapid prior to attainment of diffusion equilibrium following the intravenous dose. Thereafter the shape of the curve corresponds to that observed in curve *D* for orally administered SETD.

Except for a slightly more rapid absorption than average, the composite data of Fig. 3 constitute a fairly accurate representation of the kinetics of SETD absorption, distribution, and excretion in the average healthy adult human subject of these studies. These data illustrate the following facts: drug disappearance rates, after absorption and diffusion equilibrium, are similar for orally or intravenously administered SETD, and from blood concentration or urinary excretion data; sequestering or storage does not occur to any detectable extent; and absorption is efficient, with absorption rate being very rapid in comparison with excretion rate.

The data as portrayed in Fig. 3 have an application to dosage form design, determination of dosage regimens, and in the evaluation of blood and urine concentration data obtained from administration of conventional and sustained release dosage forms. For example, it is clinical practice to prescribe fixed doses of some chemotherapeutic agents at equally spaced times. Knowing the standard performance indices (1) it may be possible to calculate the "steady state blood concentrations" resulting from the oral administration of a given drug according to such a prescribed regimen, possibly minimizing time, effort, and cost in acquiring such data in pharmaceutical product development. Also, such data may be of value in the design of oral sustained release dosage forms because any change in drug release will necessarily influence absorption, excretion, and tissue concentrations of the drug. Theoretically, knowing the specific velocity constant for drug elimination from blood or urine drug concentration data coupled with a knowledge of *in vivo* drug absorption and distribution characteristics, should assist in the determination of the dose required to attain a certain tissue concentration or effect, and the absorption or release rate required to maintain it. These and other applications to SETD will be discussed further in future publications from these laboratories (10).

SUMMARY

1. Oral administration of SETD to humans results in rapid absorption of the drug, generally giving measurable blood concentrations within twenty minutes and peak therapeutic concentrations within one to three hours.

2. After absorption is complete and diffusion equilibrium is attained, orally administered SETD is excreted in a manner and at a rate comparable to an intravenous dose. Urinary excretion of SETD is rapid when contrasted with some other clinically available sulfonamides, an average of 85 per cent being excreted in forty-eight hours.

3. The per cent distribution volume, determined from blood concentration data after oral doses was 20 per cent, a value corresponding closely to that observed following intravenous doses.

4. These and previously reported data for this drug permit a delineation and interpretation of the kinetics of drug absorption, distribution, and excretion.

5. The importance of these data and interpretations to design problems of standard oral dosage forms and oral sustained release dosage forms and regimens is discussed.

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Pharmacological Studies on Bis-(2-Methyl-2-Nitropropyl) Sulfite*

By FRED T. GALYSH, GEORGE H. BERGMAN, and CLIFTON E. MILLER

Pharmacological studies on bis-(2-methyl-2-nitropropyl) sulfite indicate that this compound has sedative, hypotensive, and antispasmodic activity. The drug also possesses a negative chronotropic and negative inotropic action upon the frog heart. The results of a chronic toxicity study are also reported.

A PREVIOUS PUBLICATION (1) has reported the preparation of bis-(2-methyl-2-nitropropyl) sulfite, an aliphatic nitro compound which contains sulfur in an intermediate valence. This publication also reported the *in vivo* inhibition of this compound on Sarcoma 180 in mice, as well as the results of a toxicity study in week-old chicks. The apparent need for a more detailed pharmacological study of this type of compound, prior to synthesis of possible analogues, prompted the work to be reported herein.

EXPERIMENTAL

Bis-(2-methyl-2-nitropropyl) sulfite (hereafter designated BMNS) was synthesized and purified in our laboratory of pharmaceutical chemistry. Its identity was established from melting point data and solubility characteristics. The insoluble nature of BMNS required its preparation for injection in the form of a suspension in oil-in-water emulsions containing either cholesterol (2.5%) or lecithin (0.1%) as emulsifying agents. For the chronic toxicity study, an aqueous suspension containing 5% BMNS and 0.1% lecithin was utilized. An aqueous suspension of BMNS in 5% acacia was used for the smooth muscle studies. It was found that 1,4-dioxane, used as a solvent by Kessler, *et al.* (1), was too toxic in the species employed in this study.

The pharmacological action of BMNS was determined in eleven unanesthetized rats and two unanesthetized cats. The drug was administered by either the oral, subcutaneous, or intraperitoneal routes. BMNS effects on the blood pressure and respiration were also determined in six rats, seven cats, and four dogs. The cats and dogs used in the blood pressure experiments were anesthetized intraperitoneally with pentobarbital sodium (35 mg./Kg.) and the rats with urethane (1 Gm./Kg., i. p.). Doses of 250 mg./Kg. of the sulfite were administered intraperitoneally, intramuscularly, or intravenously. The blood pressure responses were recorded from a cannulated carotid artery via a mercury manometer. Respiratory movements were recorded from a tambour connected to a tracheal cannula. The blood pressure effects of BMNS in rats injected intraperitoneally were recorded from a cannulated carotid artery through a special recording mercury manometer (2).

A 1:4000 aqueous suspension of BMNS was applied directly to the frog heart enclosed within a small cup. Contractions of the heart were recorded through a heart muscle lever upon a smoked kymograph drum. Six *Rana pipiens* frogs, weighing 50 to 60 Gm., were employed in this study. The effect of BMNS (1:4000 aqueous suspension) on electroshock threshold of the frog gastrocnemius-sciatic preparation was also determined by conventional methods.

Five intestinal strips from three rabbits, two intestinal strips from a guinea pig, and three intestinal strips from two rats were utilized for studies on smooth muscle. The preparations were suspended in a 100-ml. muscle chamber containing either Tyrode's or Locke-Ringer's solution maintained at a temperature of 37.5–38.0°. Tyrode's solution was used for the guinea pig intestine, while the rabbit intestine was placed into Locke-Ringer's solution. The muscle responses were recorded by a long, writing lever on a smoked kymograph. The effects of a 1:40,000 aqueous suspension of BMNS were

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thus studied. The action of the sulfite on tracheal musculature was determined by the method described by Castillo and de Beer (3); tracheal chains from two guinea pigs were prepared for these experiments. The action of the sulfite (1:40,000 aqueous suspension) was also studied on the smooth muscle of the thoracic aorta of either guinea pig or bovine origin. The method of Furchgott and Bhadrakom (4) was employed.

Chronic toxicity studies of BMNS were made in both male and female albino rats of different ages by the daily intraperitoneal injection of 250 mg./Kg. for a period of twenty-eight days. At this time the animals were sacrificed by concussion and exsanguination and the internal organs examined grossly for evidence of pathologic processes.

RESULTS

Action in Unanesthetized Rats and Cats.—The drug was administered in a dosage of 250 mg./Kg. to rats and cats. Subcutaneous injection of the sulfite appeared to have little or no effect upon the rat. Oral administration of a similar dosage resulted only in production of diarrhea in approximately one and one-half to two hours. When given intraperitoneally, however, BMNS imparts to the animal a definite mild sedation occurring twenty minutes post-injection. The rat is easily aroused and is extremely adverse to handing, responding irritably to tactile stimulation. The cat fails to exhibit a similar sedative effect upon intraperitoneal injection of 250 mg./Kg. of the drug; on the contrary, this species appears to be somewhat more excited than the control animal. The degree of stimulation, however, is not as intense as that seen with small doses of morphine in the cat.

Action in Anesthetized Animals.—In normotensive rats doses of 250 mg./Kg. intraperitoneally lowered the blood pressure for periods up to three hours. The maximum average depressor response in all the normotensive animals utilized was approximately 40 mm. Hg with rapid onset and a duration of action in excess of two and one-half hours. Respiratory rate and amplitude were little affected throughout all of the blood pressure experiments. Normotensive cats and dogs exhibited a depressor response similar to that seen in rats (Fig. 1). The hypotension in the larger animals, however, was more persistent, lasting from three to five hours. The heart rate was decreased 5–8% below normal control levels; this effect was maximal one hour post-injection with gradual recovery to a normal rate. The depressor response in cats and dogs was evident only after intraperitoneal administration; intramuscular and intravenous injection appeared to be completely ineffective. Post-mortem examination of the animals revealed no evidence of unabsorbed BMNS in the peritoneal cavity. Similar examination of intramuscular sites, however, indicated that the sulfite was very poorly absorbed by this route.

Data obtained from rats and dogs with spontaneous, abnormally high, mean arterial pressure indicate that the drug is without effect as an antihypertensive agent. A depressor response could not be elicited if the pre-injection mean arterial pressure exceeded 150 mm. Hg.

Action on Frog Heart *in Situ*.—A 1:4000 aqueous suspension of BMNS produced a progressive slowing and decrease in amplitude of contraction of the frog heart *in vivo* (Fig. 2) with distinct evidence of partial A-V block of amplitude. There was no recovery from this effect. The effect was only partially prevented by pre-treatment of the heart with atropine.

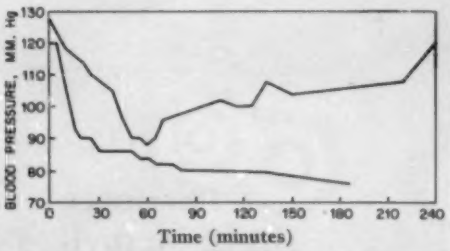

Fig. 1.—Upper curve—female cat, 4.2 Kg., pentobarbital sodium anesthesia, 35 mg./Kg., i. p. Note: Bis-2-methyl-2-nitropropyl sulfite, administered i. p. at zero time, causes a gradual, but marked fall of blood pressure with return toward normal occurring in four hours. The heart rate was decreased by approximately 6% at the end of one hour with a gradual recovery. The respiratory rate was little affected. Lower curve, female rat—0.315 Kg., urethane anesthesia, 1 Gm./Kg., i. p. Note: BMNS, administered i. p. exerts a hypotensive effect, which is rapid in onset and persists longer than two and one-half hours. At the end of this time the respiratory rate continued at the normal level of 72 per minute.

Action on Muscle-Nerve Preparation of Frog.—BMNS, (1:4000 in Ringer's solution) applied to the muscle-nerve preparation for fifteen minutes, has no effect on threshold stimuli required to produce contraction of the gastrocnemius muscle either by stimulation of the sciatic nerve or the muscle directly.

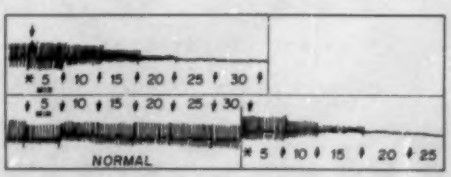

Fig. 2.—Effect of BMNS on the frog heart *in situ*. Administration of the drug is indicated by asterisk. Note the gradual slowing and decrease in amplitude of contraction. Results from two frogs were recorded simultaneously. The frog forming the lower tracing served as a control; the latter part of this tracing (beginning at asterisk) was formed by a confirmatory animal.

Action on Isolated Smooth Muscle.—A 1:40,000 suspension of BMNS reduced the tone and amplitude of normally contracting intestinal smooth muscle strips. Spasms of the small intestine induced with 1:100,000 dilutions of methacholine were diminished by 1:40,000 aqueous suspension of BMNS. The muscle did not respond maximally again to methacholine until four doses of this drug were applied at intervals of ten minutes. The muscle was thoroughly washed two minutes before each subsequent addition of methacholine (Fig. 3). BMNS (1:40,000) also reduced the spasm of small intestine of guinea pigs, rats, and rabbits induced by varying concentration of barium chloride. The compound apparently was without effect on histamine-induced constriction of isolated tracheal smooth muscle (tracheal chain). BMNS (1:40,000) caused a complete relaxation of aortic smooth muscle placed in a state of moderate contraction by epinephrine (1:100,000,000 dilution).

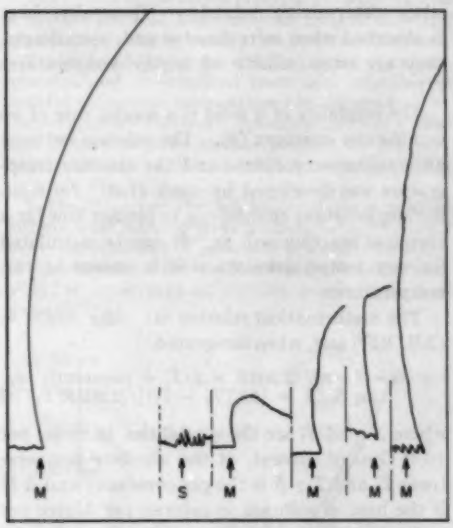

Fig. 3.—Action of BMNS on a strip of rabbit's isolated small intestine suspended in a 100-ml. bath of Locke's solution at 37.5°. Note: A 1:40,000 suspension of BMNS (S) blocked the stimulant effect of 1:100,000 solution of methacholine (M); also, four doses of methacholine ten minutes apart were required before the BMNS action was completely overcome. The muscle was washed two minutes before addition of drugs.

Chronic Toxicity in Rats.—Chronic administration of BMNS to rats for four weeks appeared to induce no symptoms of toxicity which could be grossly observed in the intact animal. There was some exophthalmos observable up to one hour after injection. Red blood cell counts made at the termination of the study revealed that the drug depressed erythrocytic counts from 40–50% below normal control levels in more than one-half of the treated animals. Post-mortem examination indicated that there was some diffuse and pin-point hemorrhage of the lung in all the rats. There was much evidence of adhesion formation between the abdominal viscera and the serous layer of the peritoneal wall. Little unabsorbed drug occurred in the peritoneal cavity; however, deposition of the drug in the splenic tissue served as evidence of the activity of the phagocytic cells of the spleen and the minimal solubility of BMNS in body fluids.

DISCUSSION

Bis-(2-methyl-2-nitropropyl) sulfite, administered intraperitoneally, exerts a hypotensive action in anesthetized, normotensive rats, cats, and dogs with little effect on respiration and only minimal depression of cardiac rate. The compound appeared to be totally ineffective as an anti-hypertensive agent. Depression of the frog heart occurs when BMNS is applied directly; atropine is only partially effective in preventing the negative inotropic and chronotropic effects of the sulfite. Data from experiments on smooth muscle indicate that BMNS has a direct depressant action on smooth muscle organs, which may account for the depressor action in dogs, cats, and rats. Its slight depressant action on heart rate in cats and dogs probably does not account for the profound depressor action. It is not clear why the drug has a depressor action only when administered by the intraperitoneal route as opposed to other routes utilized in the study. It is a fact, however, that the major veins of the abdomen, because of their rather free mobility in the abdominal cavity, have somewhat greater ability to relax and to contract than do the veins of the remainder of the body, which are surrounded by solid structures. It may be postulated that BMNS, in intimate contact with these veins relaxes their musculature, resulting in a pooling of blood in these vessels and thus reducing peripheral mean arterial pressure. Further experimentation is necessary to confirm this hypothesis, as well as to investigate more fully the possibility of adrenergic blockade action.

It would appear that BMNS should have some merit as a gastrointestinal antispasmodic in view of its potency. Its extreme insolubility and apparent lack of absorption from the gastrointestinal tract, however, detract from its therapeutic efficacy.

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A Solubility Study of the Boric Acid-Water-Sorbitol System at Various Temperatures*

By JOHN J. SCIARRA,† JOHN AUTIAN,‡ and NOEL E. FOSS§

A study of the effect of various concentrations of sorbitol and temperature on the solubility and heat of solution of boric acid in water has been made. This system was studied at temperatures of 21°, 25°, 30°, 40°, 50°, and 60°. Concentrations of sorbitol ranging from 0 to 70 per cent by weight sorbitol in increments of 5 per cent were utilized in this study. The results of these experiments showed the relationship between (1) solubility of boric acid and (2) temperature and concentration of sorbitol. A phase diagram of the boric acid-water-sorbitol system is given. The heat of solution for the various systems was calculated. The specific gravity of all solutions was determined by the pycnometric method.

BORIC ACID is soluble to the extent of about 5% by volume in water or 1:18 at 25° (1). This limited solubility of boric acid in aqueous solution has prompted many workers to investigate the effect of various solvents and other substances on the solubility of boric acid (2–5). A recent paper by Sciarra, Autian, and Foss reported that a 70% by weight sorbitol solution increased the solubility of boric acid from the above to 18.66% by weight or 1:3.45 at 25° (6). This represented a significant increase in the solubility of boric acid and seems worthy of further study; therefore, an investigation dealing with the solubility of boric acid in various concentrations of sorbitol and at various temperatures was made. From the data, the heat of solution of boric acid was evaluated and the application of the van't Hoff equation to this system was studied.

Boric acid reacts with sorbitol in solution to form a boric acid-sorbitol complex. Upon analysis of this solution, three components can be detected: boric acid, water, and sorbitol. This multicomponent system can be represented graphically by means of a phase diagram and plotted on triangular graph paper. This diagram is useful for this system since the amount of sorbitol needed to dissolve .. given amount of boric acid can readily be ascertained from the phase diagram.

Temperature is an important consideration when determining solubility. Generally, heat is absorbed when salts dissolve and, accordingly, they are more soluble at higher temperatures (7).

The solubility of a solid is a special case of an equilibrium constant (8). The relation between an equilibrium constant and the absolute temperature was developed by van't Hoff. An equilibrium constant enables one to predict how far a chemical reaction will go; it can be calculated for any temperature when it is known at two temperatures.

The mathematical relation is: $(d\ln S/dt) = (\Delta H/RT^2)$ and, when integrated,

$$\log S = (-\Delta H/2.303R \times 1/T + \text{constant}), \text{ or,}$$
$$\log S_2/S_1 = [\Delta H(T_2 - T_1)]/[2.303R(T_2T_1)]$$

where S_2 and S_1 are the solubilities, in moles per 1000 Gm. of solvent, at the absolute temperatures T_2 and T_1; R is the gas constant; and ΔH is the heat of solution in calories per degree per mole. In this integration ΔH is assumed to be constant, an assumption that is not entirely justified (8).

If a plot of the log S *vs.* $1/T$ yields a straight line, the slope of the line becomes equal to $-\Delta H/2.303R$. From this ΔH can readily be calculated.

EXPERIMENTAL

Solutions containing 0% sorbitol to 70% sorbitol by weight were prepared by diluting Sorbo® solution,[1] with the necessary amount of water. Each of these solutions was neutralized to phenolphthalein by adding a few drops of sodium hydroxide solution to produce a faint pink color. The solution was then placed in contact with an excess of finely divided crystalline boric acid, U. S. P., in a solubility tube. The solubility tube was then placed in the thermostat and stirred constantly for twenty-four hours. After stopping the stirring motor and allowing the undissolved particles to settle to the bottom, 2 25-ml. portions of the clear supernatant liquid were removed, placed into tared beakers, and quickly

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Adapted from the manuscript submitted by John J. Sciarra which received honorable mention in the 1957 Lunsford Richardson Pharmacy Awards competition.

† Present address: Assistant Professor of Pharmaceutical Chemistry, St. John's University, College of Pharmacy, Brooklyn, N. Y.

‡ Present address: Assistant Professor of Pharmacy, University of Michigan, College of Pharmacy, Ann Arbor.

§ Dean and Professor of Pharmacy.

[1] Seventy per cent sorbitol, Atlas Powder Co., Wilmington, Del.

weighed. The amount of boric acid in these solutions was determined by assaying the solution according to the U. S. P. assay for boric acid (1).

At the same time, a 25-ml. Gay-Lussac specific gravity bottle was filled with the saturated solution of boric acid in the sorbitol solvent and the specific gravity determined by the pycnometric method (9). The specific gravity of the sorbitol solvent alone was also determined.

The solubility of boric acid and specific gravity of each of the above solutions were determined at 21°, 25°, 30°, 40°, 50°, and at 60° ± 0.1°.

RESULTS

The results of the above experiments were treated in several different ways in order to best show the relationship existing among the various solutions.

Tables I and II give the specific gravity of sorbitol solutions and of saturated boric acid solutions in sorbitol at various temperatures as determined by the pycnometric method. Specific gravity of a solution of boric acid in sorbitol solution *vs.* per cent weight sorbitol at 21° and 60° was plotted and is shown in Fig. 1.

Table III gives the solubility of boric acid in sorbitol solutions at various temperatures. Figure 2 shows the solubility of boric acid in sorbitol solutions at various temperatures while Fig. 3 shows the solubility of boric acid at various concentrations of sorbitol.

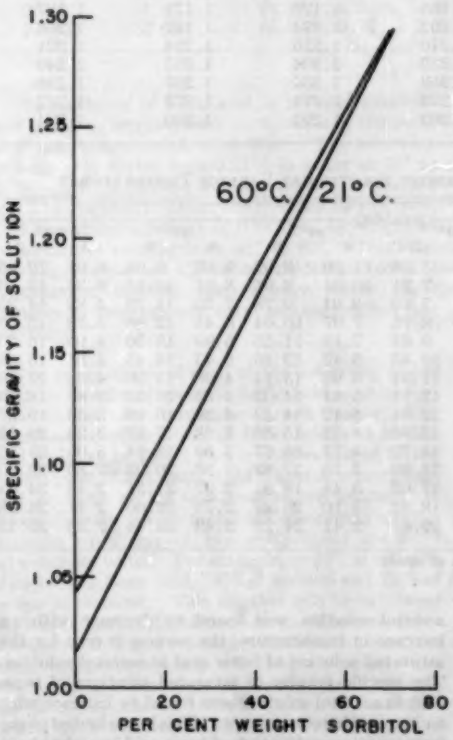

Fig. 1.—Isotherm curves showing specific gravity of saturated solution of boric acid in sorbitol.

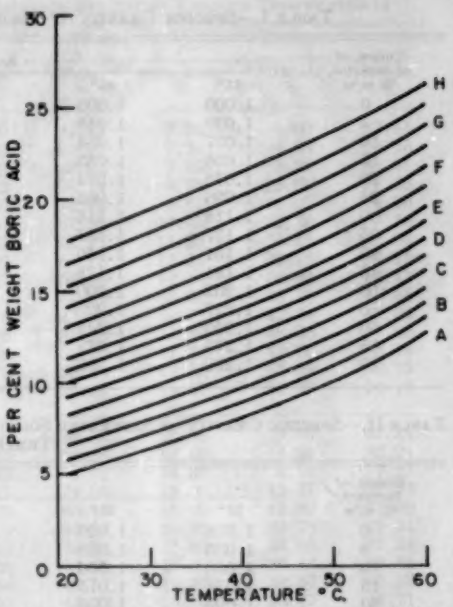

Fig. 2.—Effect of temperature upon the solubility of boric acid in sorbitol solution. A—zero, B—10, C—20, D—30, E—40, F—50, G—60, H—70, per cent weight sorbitol.

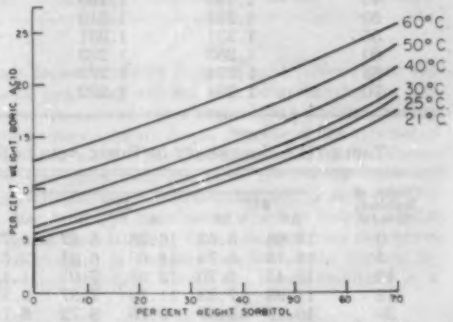

Fig. 3.—Isotherm curves showing solubility of boric acid in sorbitol solution.

The composition of various solutions consisting of boric acid, sorbitol and water is given in Table IV. The results at 25°, 40°, and 60° were plotted on triangular graph paper and are shown in Fig. 4.

Figure 5 shows the relationship between the log of boric acid solubility in moles per 1000 Gm. of solvent and reciprical of absolute temperature. From this graph the slope was determined from which ΔH was calculated. Then, by substituting these values of ΔH in van't Hoff's equation the solubility of boric acid was calculated at 35° and compared with the value obtained from Fig. 2. These results are shown in Table V.

DISCUSSION

The specific gravity of sorbitol solutions was found to increase with an increase in concentration

TABLE I.—SPECIFIC GRAVITY OF SORBITOL SOLUTIONS AT VARIOUS TEMPERATURES

Concn. of Sorbitol, % w/w	Specific Gravity					
	21°	25°	30°	40°	50°	60°
0	1.000	1.000	1.000	1.000	1.000	1.000
5	1.019	1.018	1.018	1.017	1.017	1.017
10	1.037	1.034	1.036	1.036	1.036	1.035
15	1.056	1.055	1.054	1.054	1.053	1.054
20	1.075	1.074	1.075	1.074	1.074	1.073
25	1.096	1.095	1.095	1.094	1.093	1.093
30	1.118	1.116	1.115	1.115	1.115	1.114
35	1.137	1.137	1.135	1.134	1.133	1.133
40	1.151	1.150	1.150	1.148	1.147	1.157
45	1.181	1.178	1.179	1.177	1.176	1.179
50	1.202	1.200	1.200	1.198	1.195	1.200
55	1.227	1.225	1.226	1.224	1.224	1.226
60	1.250	1.249	1.248	1.247	1.244	1.251
65	1.275	1.273	1.274	1.272	1.271	1.279
70	1.298	1.298	1.289	1.297	1.295	1.291

TABLE II.—SPECIFIC GRAVITY OF SATURATED SOLUTION OF BORIC ACID IN SORBITOL SOLUTION AT VARIOUS TEMPERATURES

Concn. of Sorbitol, % w/w	Specific Gravity					
	21°	25°	30°	40°	50°	60°
0	1.018	1.020	1.023	1.029	1.036	1.046
5	1.036	1.038	1.041	1.047	1.053	1.061
10	1.056	1.056	1.059	1.064	1.071	1.079
15	1.074	1.075	1.077	1.082	1.084	1.096
20	1.095	1.095	1.096	1.101	1.108	1.114
25	1.113	1.114	1.116	1.120	1.124	1.131
30	1.134	1.134	1.140	1.140	1.145	1.150
35	1.152	1.152	1.154	1.157	1.163	1.167
40	1.165	1.164	1.166	1.170	1.171	1.187
45	1.190	1.190	1.192	1.194	1.197	1.206
50	1.211	1.210	1.210	1.210	1.214	1.221
55	1.231	1.231	1.232	1.234	1.235	1.240
60	1.253	1.252	1.252	1.254	1.257	1.258
65	1.274	1.272	1.272	1.271	1.273	1.272
70	1.294	1.292	1.293	1.291	1.290	1.294

TABLE III.—SOLUBILITY OF BORIC ACID IN SORBITOL SOLUTION AT VARIOUS TEMPERATURES

Concn. of Sorbitol, % w/w	Solubility											
	21°		25°		30°		40°		50°		60°	
	A[a]	B[b]	A	B	A	B	A	B	A	B	A	B
0	18.88	5.03	16.98	5.57	14.73	6.36	11.36	8.09	9.03	9.98	6.91	12.65
5	16.13	5.74	14.60	6.31	12.65	7.21	10.01	8.95	8.01	10.95	6.33	13.46
10	13.45	6.70	12.68	7.09	11.12	7.99	8.91	9.78	7.25	11.76	5.82	14.25
15	11.98	7.33	11.10	7.87	9.87	8.76	7.97	10.64	6.45	12.84	5.38	15.00
20	10.19	8.37	9.75	8.72	8.74	9.63	7.13	11.55	5.93	13.56	4.90	16.00
25	9.14	9.09	8.73	9.47	7.83	10.45	6.42	12.46	5.43	14.43	4.51	16.88
30	8.17	9.88	7.71	10.42	7.04	11.31	5.93	13.14	4.98	15.26	4.15	17.80
35	7.36	10.68	7.00	11.18	6.40	12.11	5.41	14.02	4.53	16.33	3.87	18.60
40	6.88	11.22	6.62	11.63	6.01	12.64	5.12	14.53	4.36	16.69	3.56	19.57
45	6.19	12.03	5.72	12.92	5.28	13.86	4.52	15.83	3.88	17.97	3.28	20.52
50	5.63	12.87	5.20	13.80	4.84	14.72	4.17	16.67	3.59	18.94	3.00	21.76
55	5.01	13.99	4.64	14.98	4.32	15.89	3.76	17.87	3.26	20.02	2.80	22.60
60	4.44	15.28	4.15	16.17	3.91	17.02	3.44	18.91	2.97	21.31	2.45	24.63
65	3.98	16.45	3.75	17.31	3.50	18.32	3.10	20.27	2.75	22.30	2.41	24.48
70	3.66	17.43	3.37	18.66	3.19	19.47	2.41	24.20	2.48	23.74	2.25	25.65

[a] A—Solubility of boric acid expressed as ml. of solvent/Gm. of solute.
[b] B—Solubility of boric acid expressed as per cent by weight.

of sorbitol and decrease with an increase in temperature. The specific gravity of the various solutions varied from 1.000 for pure water (0% sorbitol) to 1.291 for a 70% solution of sorbitol at 60°. As shown in Fig. 1, the relationship existing between specific gravity of a saturated solution of boric acid in sorbitol solution and concentration of sorbitol is a linear function. While the specific gravity of sorbitol solution was found to decrease with an increase in temperature, the reverse is true for the saturated solution of boric acid in sorbitol solution. The specific gravity of saturated solutions of boric acid in sorbitol solution was found to increase with an increase in temperature. This is attributed to the increase in concentration of boric acid brought about by the increase in temperature.

TABLE IV.—COMPOSITION OF BORIC ACID-WATER-SORBITOL SYSTEM AT VARIOUS TEMPERATURES

Composition								
Boric Acid, %	Sorbitol, %	Water, %	Boric Acid, %	Sorbitol, %	Water, %	Boric Acid, %	Sorbitol, %	Water, %
21°			25°			30°		
5.03	0.00	94.97	5.57	0.00	94.43	6.36	0.00	93.64
5.74	4.76	89.50	6.31	4.68	89.01	7.21	4.64	88.15
6.70	9.33	83.97	7.09	9.29	83.62	7.99	9.20	82.81
7.33	13.90	78.77	7.87	13.82	78.31	8.76	13.69	77.55
8.37	18.33	73.30	8.72	18.26	73.02	9.63	18.07	72.30
9.09	22.73	68.18	9.47	22.63	67.90	10.45	22.39	67.19
9.88	27.04	63.08	10.42	26.87	62.71	11.31	26.61	62.08
10.68	31.26	58.06	11.18	31.09	57.73	12.11	30.76	57.13
11.22	35.51	53.27	11.63	35.47	52.90	12.64	34.94	52.42
12.03	39.59	48.38	12.92	39.19	47.89	13.86	38.76	47.38
12.87	43.57	43.56	13.80	43.10	43.10	14.72	42.64	42.64
13.99	47.31	38.70	14.98	46.76	38.26	15.89	46.26	37.85
15.28	50.83	33.99	16.17	50.30	33.53	17.02	49.79	33.19
16.45	54.31	29.24	17.31	53.75	28.94	18.32	53.09	28.59
17.43	57.80	24.77	18.66	56.96	24.38	19.47	56.37	24.16
40°			50°			60°		
8.09	0.00	91.91	9.98	0.00	90.02	12.65	0.00	87.35
8.95	4.55	86.50	10.95	4.45	84.60	13.46	4.33	82.21
9.78	9.02	81.20	11.76	8.82	79.42	14.25	8.58	77.17
10.64	13.40	75.96	12.84	13.07	74.09	15.00	12.75	72.75
11.55	17.69	70.76	13.56	17.29	65.15	16.00	16.80	67.20
12.46	21.89	65.65	14.43	21.39	64.18	16.88	20.78	62.34
13.14	26.06	60.80	15.26	25.42	59.32	17.80	24.66	57.54
14.02	30.07	55.91	16.33	29.28	54.39	18.60	28.84	52.56
14.53	34.19	51.28	16.69	33.34	49.97	19.57	32.57	47.86
15.83	37.88	46.29	17.97	36.91	45.12	20.52	35.77	43.71
16.67	41.67	41.66	18.94	40.53	40.53	21.76	39.13	39.11
17.87	45.17	36.96	20.02	43.99	35.99	22.60	41.47	35.93
18.91	48.65	32.44	21.31	47.21	31.48	24.63	45.22	30.15
20.27	51.32	27.91	22.30	50.51	27.19	24.48	49.09	26.43
24.20	52.96	22.84	23.74	53.38	22.88	25.65	52.05	22.30

The solubility of boric acid in sorbitol solutions was found to increase with an increase in temperature and concentration of sorbitol. The solubility of boric acid varied from 5.03% in water at 21° to 25.7% in 70% sorbitol solution at 60°. Figures 2 and 3 can be used to determine the solubility of boric acid in sorbitol solutions of 0 to 70% by weight at temperatures ranging from 21° to 60°. This relationship is important when one considers problems of formulation, stability, and storage where slight changes in temperature may seriously affect the solubility.

The composition of each of the solutions, in per cent by weight of sorbitol, boric acid, and water, was plotted on triangular graph paper. The maximum concentration of boric acid was at 60° and found to be 25.7% at a concentration of 52.1% sorbitol and 22.2% water. At higher concentrations one or more of the components precipitated. A phase diagram is very useful in determining concentrations when one or two components are fixed at a definite value. For example, at 25°, in order to dissolve 8% boric acid, 14% of sorbitol and 78% of water is required. This solution will be saturated with boric acid at this temperature. The area above the curves in Fig. 4 represents concentrations of the three components which will yield a clear solution. One or more components will precipitate in the area beneath the curves. Theoretically, a maximum solubility is reached at about 50 to 60% sorbitol (depending upon temperature) and it is believed that higher concentrations of sorbitol will actually result in a lesser solubility of boric acid. Since both the boric acid and sorbitol will be competing for the lesser amount of water present, the sorbitol, having the greater solubility, will dissolve at the expense of the boric acid. This phenomenon is further shown on the phase diagram, since the curves must approach the point representing 100% sorbitol, 0% water, and 0% boric acid, which is located at the apex of the triangle. As can be seen from the curves the increase in temperature lowers the curves, increasing the area above the curve resulting in a

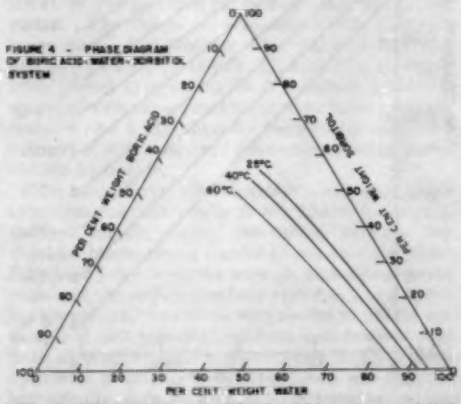

Fig. 4—Phase diagram of boric acid–water–sorbitol system.

greater area of solubility for boric acid in the sorbitol solvent.

Figure 5 shows the log of solubility *vs.* reciprocal of absolute temperature. This plot yielded a straight line from which the slope was determined. Calculation of ΔH for each of the solvents ranging from water (0% sorbitol) to 70% sorbitol, shows that ΔH decreases with an increase in concentration of sorbitol. This change indicates that less heat is involved when a mole of boric acid dissolves in 70% sorbitol as compared to dissolving in water or in a lesser concentration of sorbitol. The application of van't Hoff's equation to this system was tested and found to give acceptable results as shown in Table V. Utilizing this equation, the solubility of boric acid can be calculated at any temperature. This constant variant is important in pharmaceutical formulations since a knowledge of solubility enters into many problems.

TABLE V.—COMPARISON OF CALCULATED SOLUBILITY WITH EXPERIMENTALLY DETERMINED SOLUBILITY OF BORIC ACID

A	B	C	D	E
0	5.030	1.223	1.257	1.236
5	4.580	1.405	1.399	1.406
10	4.260	1.560	1.558	1.551
15	4.030	1.755	1.723	1.699
20	3.840	1.895	1.907	1.897
25	3.470	2.082	2.058	2.081
30	3.420	2.247	2.269	2.268
35	3.200	2.475	2.426	2.416
40	3.200	2.541	2.537	2.556
45	3.200	2.779	2.860	2.798
50	3.050	2.997	3.060	2.989
55	2.900	3.249	3.340	3.254
60	2.750	3.556	3.576	3.550
65	2.470	3.848	3.875	3.792
70	2.410	4.193	4.245	4.144

A—Concentration of sorbitol, per cent by weight. B—ΔH calculated from slope, calories/mole/degree. C—Calculated solubility at 35° when T_1 = 323° K., moles/1000 Gm. solvent. D—Calculated solubility at 35° when T_1 = 298° K., moles/1000 Gm. solvent. E—Solubility determined experimentally from Fig. 2, moles/1000 Gm. solvent.

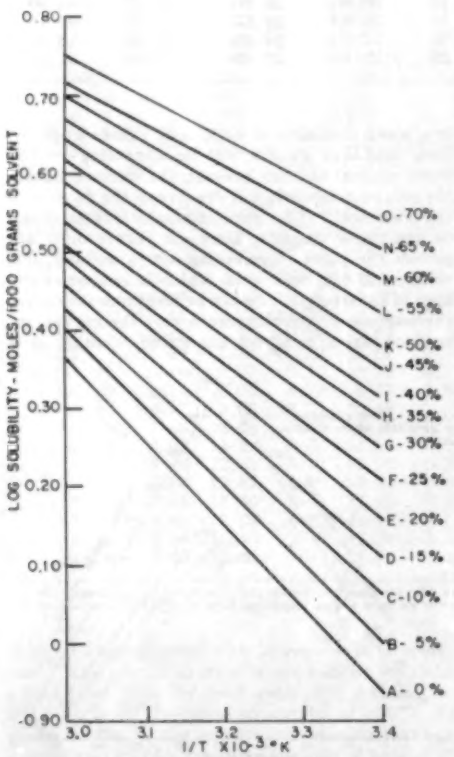

Fig. 5—Plot of log solubility of boric acid *vs.* reciprocal of absolute temperature

SUMMARY AND CONCLUSIONS

1. A solubility study of the boric acid-water-sorbitol system has been made at various temperatures.

2. The specific gravity of sorbitol solutions was determined by the pycnometric method and found to increase with an increase in concentration of sorbitol, and decrease with an increase in temperature. While the specific gravity of sorbitol solution was found to decrease with an increase in temperature, the reverse was found to be true for the saturated solution of boric acid in sorbitol solution. This was due to the increased solubility of boric acid at elevated temperatures.

3. The solubility of boric acid in sorbitol solution was determined at various temperatures and concentrations of sorbitol and found to increase with an increase in temperature and concentration of sorbitol.

4. A phase diagram of the boric acid-water-sorbitol system was determined at various temperatures. The heat of solution, ΔH, for the various systems was evaluated by plotting log of solubility of boric acid *versus* reciprocal of absolute temperature. It was found that ΔH decreased with an increase in concentration of sorbitol. The application of van't Hoff's equation to this system was tested and found to give acceptable results.

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The Distribution and Fate of Iodine in Iodopropylidene Glycerol in the Rat*

By GEORGE F. HOFFNAGLE† and ARTHUR OSOL

The distribution and fate of iodine in iodopropylidene glycerol in the rat were studied by use of the compound tagged with iodine^{131}, and comparisons were made with a similar study of tagged potassium iodide, both substances being given in a single dose of a magnitude equivalent to the therapeutic dose of potassium iodide in humans. Iodopropylidene glycerol was found to provide more prolonged though lower blood iodide levels and higher thyroidal iodine levels than an equivalent dose of potassium iodide.

THE HISTORY of systemic iodine therapy is long and confused, and the application of such therapy has traversed the gamut of diseases. In the past half century, various clinical and laboratory studies have established the value, in greater or less degree, of iodine therapy in many disease entities. The practical problems incident to systemic iodine therapy have been chiefly those of gastric irritation caused by iodides and elemental iodine, and the onset of iodism attending continued high-level therapy.

One compound which appears to produce desirable clinical benefits without causing gastric distress or iodism is iodopropylidene glycerol (1–5), hereafter called IPG.[1] The apparent advantages of IPG prompted this investigation of its biological distribution and utilization.

The lack of gastric distress is undoubtedly attributable both to the absence of iodide ion and free iodine in the preparations of the compound and, by virtue of the stability of the compound in acid media, to the lack of release of these in the stomach.

Biological work done elsewhere indicates the compound to be devoid of demonstrable chronic toxicity at reasonable levels, and to have an LD_{50} of approximately 700 mg. per Kg. of body weight when injected intraperitoneally into mice; its intestinal absorption is equal to, or exceeds, that of Lugol's solution; and it produces the same levels of protein-bound iodine in blood as do inorganic iodides (6, 7). Its efficacy and efficiency for iodine therapy have been established.

EXPERIMENTAL

In order to determine the distribution of a single therapeutic dose of iodine, the approximate equivalent for rats of the amount of iodine represented in the average human dose of potassium iodide (300 mg.) was used, this amounting to 1 mg. of iodine per rat, given as 1.3 mg. of potassium iodide or 2 mg. of IPG. The potassium iodide, tagged with 100 microcuries of I^{131}, was given in 1 ml. of water. The IPG, tagged with variable radioactivity depending on the tagging achieved, was given in 1 ml. of 60% v/v glycerin solution orally, or 20% v/v glycerin solution intravenously. No evidence of damage to thyroid tissue by radiation was observed either macroscopically or by gland weights in any experiment.

Young male Wistar rats from 250 to 300 Gm. in weight were brought to iodine equilibrium by feeding solely on Purina Dog Chow for two weeks prior to the experiments. Immediately after giving the dose orally using a feeding needle, the rats were placed in metabolism cages mounted on shallow funnels. The funnels were fitted with paper toweling with the center hole open, the toweling being treated with sodium hydroxide solution. The feces could thus be collected in sodium hydroxide solution under the funnel, while the alkali on the paper achieved immediate absorption of all urine and prevented loss of iodine by decomposition to elemental form.

Five rats were used for each time period in each experiment. The rats were sacrificed by ether anesthesia at the specified times and immediately dissected. The tissues were studied by the following procedures. The thyroids were digested individually by the Blau procedure (8), involving an eight-hour period of refluxing in 8% barium hydroxide solution, with subsequent separation into a thyroxin fraction and a non-thyroxin fraction by *n*-butanol extraction of the digested solution containing added sodium hydroxide.

The lungs, liver, spleen, kidneys, stomach, small intestines and contents, large intestine and contents, skeletal muscle, brain, fat (from between the shoulder blades, being careful to use only the white fat), knee joint (with the bone on each side cropped close and the muscle carefully removed), and eye—both whole and lens only, were pooled to obtain one sample of each type of tissue from each five rats, and were digested at boiling temperature in 4*N* sodium hydroxide solution. The whole blood was counted individually, directly as a 1:1 dilution in anticoagulant citrate solution U. S. P., since Owen and

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This investigation was supported in part by a grant from Henry K. Wampole & Co., Inc., Philadelphia, Pa.
† Present address: 86 Forest Ave., Verona, N. J.
[1] Preparations of IPG used in this study were supplied by Henry K. Wampole & Co., Inc., Philadelphia, Pa.

Power (9) have shown that the blood iodide distribution is 0.565 times as high in the cells as it is in the plasma. The bone was digested in hot sulfuric acid-chromic acid solution, thereby converting the iodine to iodate. All digested solutions were brought to definite volumes in volumetric flasks, and 2-ml. aliquots were counted in a lead-shielded counting chamber using an end-window, organic-quenched, Geiger-Mueller counter tube and a Tracerlab Superscaler. All counts were corrected for dead time, background, to RaDEF standard, for decay, and for dilution. The counts per minute obtained for the *n*-butanol samples must be reduced by 15% for comparison with the aqueous samples, due to the difference in self-absorption in the sample caused by the change of solvents. Calculations were made in terms of counts per total tissue and/or counts per Gm. of tissue, as indicated. These were then converted to per cent of total dose.

No attempt was made to do more than follow the distribution of the iodine in this series. Preliminary experiments indicated that the iodine in IPG is completely converted to inorganic iodide by both of the alkaline digestive procedures used in the analysis.

The results represent the average for 10 rats at each time interval where standard deviation is given, or the average for five rats where standard deviation is not given, and for five rats for all values at the seventy-two-hour period. The important results, given in Table I, are on a wet tissue basis. The values for kidneys, liver, lungs, skeletal muscle, spleen, joint, bone, fat, eye, lens, and brain were proportionately lower than, and followed closely, the blood levels.

Chromatographic Study of Thyroid, Blood, and Urine 24 Hours after Administration of IPG or Potassium Iodide.—Rats administered the equivalent of 1 mg. of iodine, as tagged IPG or potassium iodide, given orally or intravenously, were sacrificed at twenty-four hours and blood, thyroid, and urine samples were obtained. The blood was obtained by excising the heart, and immediately diluted with an equal volume of anticoagulant citrate solution; the radioactivity of a 2-ml. sample was determined, and the remainder centrifuged to obtain a plasma sample. The excised gland was immediately weighed, extracted with *n*-butanol by a modification of the Gross and Leblond (10) method, using a revolving Teflon pestle in a Pyrex tube mortar, 0.1 ml. of water added to accomplish the contusion, followed by 1 ml. of *n*-butanol to complete the disintegration and to extract the iodinated contents. *n*-Butanol was shown to be a good solvent for IPG as well as all normal thyroid iodine compounds (10). This process yielded 1 ml. of solution, from which the solid sediment was removed by centrifugation; the radioactivity of this solution was measured.

The total thyroid extract, 0.1 ml. of the 1:1 diluted plasma, and 0.1 ml. of the twenty-four-hour urine, along with radioactive sodium iodide and IPG standards, were placed on Whatman No. 1 filter paper sheets by pipetting 0.006-ml. portions on the origin spot at a time, drying each application with the aid of mild heat and a current of air. Chromatography was performed by an ascending technique, using *n*-butanol saturated with pH 7.2 phosphate buffer, for a period of approximately twelve hours. In conjunction with this phase of the study it was demonstrated: that iodide ion is trapped by plasma and exhibits little or no chromatographic movement, regardless of whether the two are brought together *in vivo* or *in vitro;* that IPG, placed on the paper first, with normal plasma afterward placed on it, migrated as expected according to the IPG standard; that IPG superimposed by urine also migrated as expected. Chromatograms were air-dried for approximately one-half hour, then scanned for radioactivity on a scanning table (54). A complete set of the chromatograms of one rat in each dose category were autoradiographed.

The chromatograms indicated that: no IPG was present in blood, urine, or thyroid at twenty-four hours; iodide ion was always present in all three tissues at twenty-four hours, regardless of whether IPG or potassium iodide was given or by what route; occasional rats yielded sufficient radioactive thyroxin-like material to be detectable, though of a very low order. This last is in contradistinction to the results of Gross and Leblond (10), who used carrier-free radioiodide which was taken up and converted by thyroid with the result that much higher radioactivity levels were obtained.

Studies on Initial Distribution of Iodopropylidene Glycerol in the Rat.—In this phase of the study selected solid tissues of rats receiving tagged compounds were homogenized in a Waring Blendor with 0.1% potassium iodide solution; and the radioactivity of aliquots of these homogenates, as well as of blood samples, was measured. Then chloroform extracts were prepared by thorough agitation of the homogenates and of the whole blood with measured volumes of chloroform. Phase separation was effected by centrifugation. Aliquot portions of the chloroform extracts were counted for an estimate of the IPG portion of the total activity previously measured. The IPG was again the I^{131}-tagged material prepared as previously described. Measurements of the radioactivity were made on 2-ml. samples enclosed in a 2-inch lead shield, using an end-window, organic-quenched, Geiger-Mueller counter tube and a Tracerlab Superscaler. The solubility ratio of IPG in chloroform-water systems and the variation of self-absorption in chloroform solutions from the self-absorption in water (19% greater self-absorption in chloroform) were taken into account.

The rats were anesthetized by administering 35 mg./Kg. of pentobarbital sodium intraperitoneally, occasionally supplemented at longer time intervals with ether anesthesia at the end of the experimental period in order to terminate the individual experiments at the specified time. Oral and intravenous routes of administration were compared in order to throw more light on the question of absorption from the gastrointestinal tract.

Sufficient paper chromatograms (11) were run on the chloroform extracts to show that activities reported for these chloroform extracts are due to IPG as such. *n*-Butanol saturated with pH 7.2 phosphate buffer solution was used as the solvent. Results of these studies are shown in Figs. 1 and 2, and Table II.

TABLE I.—RESULTS OF I^{131} DISTRIBUTION STUDIES ON POTASSIUM IODIDE AND IPG, AFTER SINGLE ORAL DOSE CONTAINING 1 MG. OF IODINE

Tissue	Potassium Iodide		Iodopropylidene Glycerol	
	Dose/Gm. of Tissue, %	Dose in Entire Tissue, %	Dose/Gm. of Tissue, %	Dose in Entire Tissue, %
Distribution 4 Hr. after Administration				
Blood, whole	0.31 ± 0.03	5.2[a] ± 0.5	0.19 ± 0.05	3.3[a] ± 0.9
Feces, total				
Thyroid, thyroxin	0.025 ± 0.025	0.0004 ± 0.0005	0.68 ± 0.5	0.016 ± 0.008
Thyroid, non-thyroxin	2.5 ± 0.6	0.040 ± 0.012	13.2 ± 4.4	0.25 ± 0.10
Urine, total		18.9 ± 5.7		8.1 ± 4.4
Distribution 12 Hr. after Administration				
Blood, whole	0.078 ± 0.03	1.4[a] ± 0.5	0.075 ± 0.04	1.6[a] ± 0.9
Feces, total		28.1 ± 11.		22.4 ± 13.4
Thyroid, thyroxin	0.44 ± 0.25	0.011 ± 0.006	5.4 ± 2.1	0.090 ± 0.040
Thyroid, non-thyroxin	5.9 ± 2.5	0.16 ± 0.08	55.0 ± 14 0	0.91 ± 0.23
Urine, total		49.1 ± 10.		29.1 ± 9.2
Distribution 24 Hr. after Administration				
Blood, whole	0.0091 ± 0.0015	0.15[a] ± 0.03	0.051 ± 0.012	0.92[a] ± 0.15
Feces, total		22.1 ± 10.		29.7 ± 6.7
Thyroid, thyroxin	4.0 ± 0.8	0.016 ± 0.003	5.9 ± 2.7	0.098 ± 0.042
Thyroid, non-thyroxin	28.5 ± 3.0	0.58 ± 0.09	52.9 ± 17.	0.83 ± 0.47
Urine, total		67.4 ± 13.1		35.7 ± 6.4
Distribution 48 Hr. after Administration				
Blood, whole	0.0010 ± 0.00015	0.018[a] ± 0.0028	0.0083 ± 0.0045	0.15[a] + 0.08
Feces, total		22.2 ± 11.1		39.0 ± 14.3
Thyroid, thyroxin	1.67 ± 0.33	0.034 ± 0.014	6.35 ± 3.3	0.11 ± 0.06
Thyroid, non-thyroxin	15.0 ± 2.9	0.30 ± 0.15	52.9 ± 26.	0.93 ± 0.40
Urine, total		64.6 ± 12.5		53.9 ± 15.
Distribution 72 Hr. after Administration				
Blood, whole			0.0012 ± 0.0019	0.021[a] ± 0.035
Feces, total				37.7 ± 9.8
Thyroid, thyroxin			3.8 ± 2.0	0.065 ± 0.034
Thyroid, non-thyroxin			28.2 ± 19.0	0.47 ± 0.30
Urine, total				55.0 ± 10.1

[a] Based on average blood volume of 6.7 ml. per 100 Gm. in rats.

DISCUSSION

The absorption of 2 IPG from the gastrointestinal tract of the intact rat was found to proceed to the extent of 77% at the end of two hours, (Fig. 2), allowing the conclusion that the compound is well absorbed from an oral dose. It is also shown that the gastrointestinal tract and tissues remained substantially free of iodide ion during most of this period, indicating that no significant decomposition of 2 IPG, with release of its iodine component, occurred. Since the stomach and intestinal tissues were included in this study, it is considered that the iodine is transported to the blood stream in organic form. The appearance of iodide ion at the one hundred-twenty-minute period, whereas none was observed at the ninety-minute

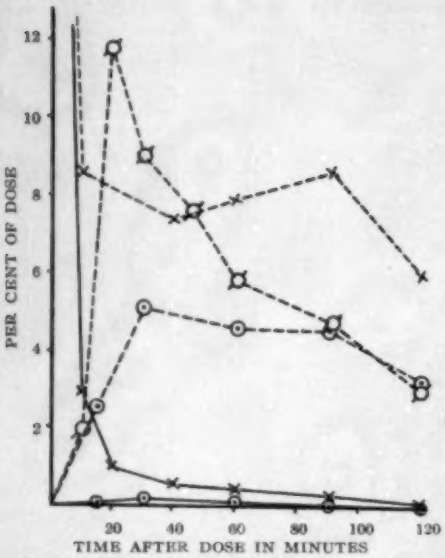

Fig. 1.—Comparison of whole blood content of total iodine and of iodine in IPG following oral (○) and intravenous (x) doses of IPG, and of iodine following oral potassium iodide (ϕ). Broken lines represent total iodine, solid lines represent iodine in IPG.

TABLE II.—PER CENT OF DOSE IN RAT ORGANS AFTER INTRAVENOUS INJECTION OF 2 MG. OF IODOPROPYLIDENE GLYCEROL, AVERAGE VALUES OF DATA OBTAINED ON 5 RATS

Tissue	Per Gram	Per Tissue
At 30 Min. after Injection		
Blood, total iodine	0.478% ± 0.037	7.70% ± 0.60
IPG iodine	0.082	1.61
Brain, total iodine	0.233	0.424
IPG iodine	0.042	0.077
Fat, total iodine	0.291	...
IPG iodine	0.231	...
Intestine, sm., total iodine	0.945	8.22
IPG iodine	0.054	0.498
Kidneys, total iodine	0.713	1.50
IPG iodine	0.015	0.030
Liver, total iodine	0.533	5.43
IPG iodine	0.014	0.148
Lungs, total iodine	0.311	0.416
IPG iodine	0.031	0.042
Muscle, total iodine	0.274	...
IPG iodine	0.048	...
Spleen, total iodine	0.342	0.774
IPG iodine	0.019	0.042
Stomach, total iodine	0.755	2.49
IPG iodine	0.053	0.173
At 10 Min. after Injection		
Blood, total iodine	0.537% ± 0.025	8.60% ± 0.39
IPG iodine	0.155	2.48
Liver, total iodine	0.877 ± 0.046	8.07 ± 0.62
IPG iodine	0.033 ± 0.008	0.303 ± 0.058

period, is considered to be evidence of secretion of iodide ion back into the tract through the stomach or bile. The results indicated that potassium iodide is more rapidly absorbed than is IPG.

The calculated fraction in the blood of the dose of IPG was always small, even only 2% at ten minutes after the intravenous dose, and always small compared with the total blood iodine. These facts require that the IPG be efficiently and quickly removed from the blood stream, and that metabolic release of the iodine from its organic combination be commenced immediately. Direct metabolic release of the organic iodine to iodide so rapidly by the blood or organs seems contraindicated by the long iodide blood levels relative to those from potassium iodide. Absence of IPG from the urine and the long iodide levels would seem to rule out excretion.

No organ or tissue studied at any of the time intervals after administration of IPG revealed any storage of either IPG or total iodine which could be considered significantly large in relation to the dose. The fat showed an early storage of IPG significantly above the total iodine level of the blood, at the same time period (thirty minutes). Since IPG is soluble in fats, the fat might be considered a logical storage depot. However, the iodine levels in the fat were barely detectable at the four-hour period (Table I) and were not detected at longer time intervals. Such a short storage time seems incompatible with the duration of blood levels observed.

Although total iodine levels in the kidneys, small intestine, stomach, and liver thirty minutes after the dose (Table II) were significantly above that in the blood at the same time, the total-organ content of iodine in each case was insignificant in comparison with the dose, and the idea of these tissues being

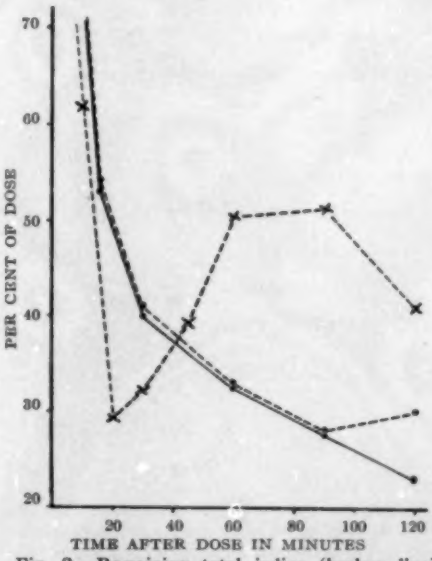

Fig. 2.—Remaining total iodine (broken line) and iodine in IPG (solid line) found in gastrointestinal tract of rats following oral doses of IPG(•) and of potassium iodide (x).

possible storage sites for IPG or iodine metabolized from it was discarded.

For its size, the thyroid gland is capable of storing a large amount of iodide. However, the amounts observed cannot be considered as representing significant storage in comparison to the total therapeutic dose, and the chromatograms did not detect any IPG.

The possibility exists that the acetal portion of IPG could be broken enzymatically to release iodinated three-carbon residues, which might escape detection as organic iodine by means of chloroform extracts because of higher water solubility. However, this same alteration of solubilities should have resulted in the appearance of separate entities in the paper chromatographic studies, but no reproducible evidence of separate entities was obtained. This was not construed to mean that the acetal ring is not broken in the metabolism of the compound. It merely gave some evidence that, if this does occur, it is probably after the iodine has been removed.

The possibility exists of the release of iodine from IPG in elemental form by a metabolic process of general distribution in the body, with consequent rapid combination with body proteins. This could be sufficiently widespread to be unobservable as a storage in a specific tissue. Also, widespread low-concentration storage of IPG itself in these tissues, with relatively slow release of iodide, is possible. These two mechanisms seem to be reasonable explanations for the data obtained in the investigation.

The investigation of iodine storage in the thyroid gland indicated that, following a single therapeutic dose, the gland was able to trap more iodine from IPG than from potassium iodide, was able to convert more of the iodide to thyroxin form from the IPG dose, and able to maintain these higher iodine levels over the comparison period studied. The investigations also revealed that the thyroid glands did not trap IPG as such, but trapped iodide released from IPG. The blood iodide levels alone, compared with those of potassium iodide, do not constitute a reasonable explanation of this picture. The possibility that this effect was the result of the use of a 60%-glycerin solution as the vehicle for IPG exists, and further work on this point is indicated.

From the prolonged duration of blood levels of IPG following a single dose, it might be presumed that in a course of therapy continuously effective thyroxin-blocking levels of blood iodide would be produced by IPG. Also, it might be considered that these levels would be maintained with IPG at a lower daily dosage than would be necessary with potassium iodide.

It might be noted here that McKnight (1, 2) produced successful involution of hyperthyroid glands and rapid cessation of thyrotoxic symptoms in the patients with large (300 mg.), once-daily, intravenous doses of IPG, without untoward symptoms.

Comparison of data obtained in this investigation with those of Broking (12), Bonanni (13), and Forbes (14), on the rate of release of iodine from iodized fatty acids and fats indicates that IPG releases iodine more rapidly than these compounds, and seems, therefore, to be intermediate between them and inorganic iodine compounds with respect to the rate of availability of its iodine.

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Book Notices

A Glossary of Mycology. By WALTER H. SNELL and ESTHER A. DICK. Harvard University Press, Cambridge, Mass., 1957. xxxi + 171 pp. 17 x 26 cm. Price $5.

This dictionary includes among its 7,000 terms some which are not strictly mycological but might be useful to students because they do occur in mycological literature. Definitions referring to the fungi includes technical, popular, vernacular, and obsolete terms; terms used in medical mycology and antibiotics; folklore, and color terms; and names of the originators of the terms. The type is small, but this is typical of books in this and related fields. It should be a useful reference for teachers, advanced students, and amateur and professional mycologists.

Dorland's Illustrated Medical Dictionary. 23rd ed. W. B. Saunders Company, Philadelphia, 1957. xvii + 1,598 pp. 17 x 26 cm. Price $12.50.

This new edition of Dorland's is bigger and, if possible, better than ever. Accuracy, authority, and quick usefulness remain the principal objectives of this reference work. It has been reviewed, revised and modernized. It is as new as the recently coined "ataractic" and "ataraxic," with their blurred difference; the respective definitions being: "Per-

taining to or capable of inducing ataraxia," and "An agent capable of inducing ataraxia." The fact that Austin Smith is responsible for modern drugs and dosage is assurance to physicians and pharmacists that expert attention has been given to this important phase of the dictionary. The longer page has permitted the addition of many words, without making the book bulkier, and the stiffer backing is a protective aid for standing usage.

Dictionary of Microbiology. By MORRIS B. JACOBS, MAURICE J. GERSTEIN, and WILLIAM G. WALTER. D. Van Nostrand Company, Inc., Princeton, N. J., 1957. 276 pp. 15 x 23.5 cm. Price $6.75.

This book contains more than 5,000 items of interest to bacteriologists, cytologists, immunologists, mycologists, biochemists, and others in related medical fields. More detailed entries are included for important culture media and methods, stains and staining, and diagnostic tests. Other entries include types of apparatus, names of chemical substances, enzymes, etc. Although some drugs are given by their generic names, and trade names are referred to the generic names, the trade names are not marked and many are omitted; e. g., under Tetracycline. . . "The group of tetracyclines, including oxytetracycline (Terramycin), chlorotetracycline (Aureomycin), tetracycline (Achromycin), Chlorotetracycline should be chloretracycline; and, for tetracycline, the trade names Panmycin, Polycycline, Steclin, and Tetracyn are omitted. However, the book should be a useful addition to the reference shelf for the many items which it includes that are not concisely described in other books; e. g., BCG Vaccine is defined, and another entry defines Calmette and Guerin's Bacillus with a cross-reference to BCG.

Heterocyclic Compounds. Vol. 6. Edited by ROBERT C. ELDERFIELD. John Wiley & Sons, Inc., New York, 1957. vii + 753 pp. 15 x 23.5 cm. Price $25.

This volume, the sixth in this authoritative and comprehensive series, covers the chemistry of the six-membered heterocycles containing two hetero atoms and their benzo derivatives, with the parent monocycles being treated separately from their benzo derivatives. In general, the attempt has been made to cover the major English and German language periodicals through 1955.

The text material is divided into chapters, contributed by specialists, under the following headings: the chemistry of the monocyclic dioxanes; benzodioxanes; sulfur analogs of the dioxanes; pyridazines; cinnolines and related compounds; phthalazine and its derivatives; pyrimidine and its derivatives; the chemistry of quinazoline; the pyrazines and piperazines, the quinoxalines; the monocyclic oxazines; the benzoxazines; thiazines and benzothiazines; phenazines, phenoxazines, and phenothiazines. The clear style, format, and type of the earlier volumes are continued in volume 6, and all that has been said in praise of this series can be repeated with much more reason now. No chemical library can be considered complete without this reference work.

The Chemistry of Plants. By ERSTON V. MILLER. Reinhold Publishing Corp., New York 22, N. Y., 1957. vii + 174 pp. 15 x 23 cm. Price $4.75.

This book presents a very limited coverage of the chemical substances found in plants, with brief descriptions of their composition, biochemical reactions, and physiological behavior. The information is presented in an interesting manner. Alkaloids are covered in a 15-page chapter which includes a discussion of the Rauwolfia alkaloids; mentioning that *R. serpentina* has been found to contain 14 substances of alkaloidal nature [more than 20 alkaloids isolated from *R. serpentina* were reported in THIS JOURNAL, **44**, 553(1955)] and noting that "Structurally reserpine contains a pentacyclic ring system." The text gives general references after each chapter, and a subject index is appended. The book should be a useful reference for those who do not wish extensive and detailed information in this field.

Experiments in Biochemical Research Techniques. By ROBERT W. COWGILL and ARTHUR B. PARDEE. John Wiley & Sons, Inc., New York, 1957. ix + 188 pp. 15 x 23 cm. Price $3.50.

This book is a laboratory text that includes thirty-seven experiments that are intended to illustrate some of the major research techniques of modern biochemistry. Physical chemical methods include: Distillation at low pressures, Countercurrent distribution, Chromatography, and Zone electrophoresis. Seventeen experiments are related to the biochemistry of enzymes. Seven experiments illustrate radioisotope tracer techniques in biochemical research. Special laboratory exercises are appended and a subject index is included. The book represents a good printing and binding job and should be a useful addition to the research library and helpful to the teacher of graduate students.

Principes de Synthèse Organique. Introduction au Mechanisme des Réactions. Edited by LÉON VELLUZ. Masson et Cie., Éditeurs, Paris, 1957. x + 599 pp. 15 x 22 cm. Price 6,000 fr.

This book (in French) considers the theoretical principles of organic chemistry as they are related to syntheses and other reactions. The first four parts of the text cover Electronic factors, Mechanisms of reactions, Steric factors, and Steric aspect of reactions. Part five includes discussions on: Degrees of oxidation, Influence of the solvent, Hammett's equation for quantitative electronic effects; and gives tabulations of: Organic groups, Radicals, and Compounds with various properties and constants. A good subject index is appended and an extensive table of contents is at the end of the book.

International Encyclopedia of Cosmetic Material Trade Names. By MAISON G. DENAVARRE. Moore Publishing Company, New York, 1957. lvi + 339 pp. 16 x 23 cm. Price $7.50.

This book is a useful compilation of information about materials and compositions in the field of cosmetics. The close relation of dermatological

formulations with many cosmetic ingredients makes the book a good reference for pharmacists. The text is divided into a main section, which lists materials by trade names; a product classification section (noted with each entry in the main listing); and a directory of manufacturers.

Organic Reactions. Vol. IX. ROGER ADAMS, Editor-in-Chief. John Wiley & Sons, Inc., New York, 1957. vii + 468 pp. 15 x 23 cm. Price $12.

This ninth volume of a series of organic reactions continues the excellent comprehensive reviews that started with volume I in 1932. Volume VIII was reviewed in THIS JOURNAL, **44**, 128(1955). Contributions of chapters by authors with special knowledge of the particular reactions is continued, as is the general plan of presentation followed in the earlier volumes.

The reactions included in this volume are indicated by the chapter headings: The cleavage of non-enolizable ketones with sodium amide, The Gattermann synthesis of aldehydes, The Baeyer-Villiger oxidation of aldehydes and ketones, The alkylation of esters and nitriles, The reaction of halogens with silver salts of carboxylic acids, The synthesis of β-lactams, and The Pschorr synthesis and related diazonium ring closure reactions. The reference value of the work is aided by tabulations of compounds that have been prepared by or have been involved in a particular reaction. The text is documented with many references to the literature and a subject index is appended. Teachers, advanced students, synthetic chemists, and libraries should have access to this book, and those who have used the earlier volumes will welcome this addition to the series.

Apotekens Register över Standardförpackade Läkemedel 1957. Edited by Apotekarsocietetens Laboratorieavdelning. Victor Pettersons Bokindustri Aktiebolag, Stockholm, Sweden, 1957. xix + 481 pp. 17 x 23 cm.

This useful reference for proprietary drugs, particularly those of Scandinavian manufacture, is published annually with paste-in supplements made available during the year. In addition to its value in a reference library, the book is a beautiful example of the art of printing and binding.

Phosphorus and Fluorine, The Chemistry and Toxic Action of Their Organic Compounds. By BERNARD CHARLES SAUNDERS. New York: Cambridge University Press, New York, 1957. xiii + 231 pp. 14 x 21.5 cm. Price $5.

This monograph is directed mainly to the advanced student of chemistry and the industrial chemist, particularly those who manufacture and handle toxic organic compounds of phosphorus and fluorine. These compounds find applications as chemical warfare agents, as constituents of agricultural insecticides, in enzyme system investigations, and as medicinal agents. The author writes with authority, particularly about di-isopropyl phosphoro-fluoridate (D. F. P.), a typical "nerve gas," the discovery of which is accredited to him.

Particularly interesting to pharmacists is Chapter X which covers esterase activity and medical aspects. The latter includes the use of D. F. P. in postoperative paralytic ileus and its effect in myasthenia gravis, and the radiosensitizing effect of organic phosphates in the radiotherapy of malignant tumors. A concise statement on first-aid treatment for nerve-gas poisoning stresses the use of atropine injection (2 mg.) repeated at intervals and artificial respiration.

The usefulness of the monograph is enhanced by having cited references as footnotes in the text. Added appendixes include methods of determination of fluorine in organic compounds and a table of properties of typical fluoro compounds. A general bibliography and a subject index are included. The format, type, and binding of the book are first rate. Medical, pharmaceutical, and chemical libraries should add this monograph to their collections.

Regulation and Mode of Action of Thyroid Hormones. Ciba Foundation Colloquia on Endocrinology. Edited G. E. W. WOLSTENHOLM and ELAINE C. P. MILLAR. Little, Brown and Company, Boston, 1957. xii + 327 pp. 13.5 × 20.5 cm. Price $8.50.

The papers compiled in this volume attempt to review the developments during the last ten to fifteen years on concepts of regulation of the thyroid gland and the character and mode of action of its hormones. The subjects presented include: Hypothalamus-pituitary-thyroid relationships; Regulation of thyroid activity; Influence of the central nervous system on the control of thyrotrophin secretion; Pituitary cytology and thyrotrophic hormone secretion; Factors influencing the thyroidal iodide pump; the "feed-back" hypothesis of the control of thyroid function; Hormone synthesis in the iodine-deficient thyroid gland; Enzymic aspects of thyronine metabolism and its iodinated derivatives; the metabolism of thyroid hormones by kidney and the biological activity of the products; The distribution and metabolism of thyroid hormones; Hepatic regulation of thyroxine metabolism; Thyroid hormones at the peripheral tissue level: metabolism and mode of action; Cellular actions of thyroxine and similar compounds; some observations of the clinical effects of triiodothyroacetic acid; and The influence of the thyroid gland upon immune responses of different species to bacterial infection. References are given after each paper and the appended subject index adds to the reference value of this volume.

Travaux des Laboratoires de Matière Médicale et de Pharmacie Galènique de la Faculté de Pharmacie de Paris. Vol. XLI, 1956. Edited by M.-M. JANOT and R. PARIS. Vigot Frères, Éditeurs, Paris, 1957. 15.5 x 23.5 cm. 479 pp.

This book (in French) is a compilation of original articles and theses on the subjects: Auto-injectable ampuls of the military health service; Contribution to the study of the alkaloids and flavonoids of some indigenous Genistas with the aid of electrophoresis; Studies on some African Rutaceae with alkaloids of the Fagara family; Contribution to the study of *Aphloia* theaeformis; Identification of drugs and control of medicaments of vegetable origin by paper

chromatography. Abstracts of other articles are included in the poorly printed, paper-back volume. No index is given.

Catalysis in Practice. Edited by CHARLES H. COLLIER. Reinhold Publishing Corporation, New York, 1957. v + 153 pp. 15 x 18.5 cm. Price $3.95.

Actual methods, economics, and problems of catalysis in the chemical process industries are described. Included are chapters on catalyst choice and commercial preparation; fixed bed systems and moving bed processes; economics of catalyst use; operating problems in processing; and an appraisal of trends and prospects in catalysis.

The Clinical Application of Antibiotics. Vol. III, *Chloramphenicol and the Tetracyclines.* By M. E. FLOREY. Oxford University Press, New York, 1957. ix + 393 pp. 15 x 25 cm. Price $19.50.

This volume is a continuation of the author's earlier work "The Clinical Application of Antibiotics—Penicillin" which was published in 1952 and which must now be considered as Volume I of this series. The aim of this book is to evaluate critically the data on which the present use of chloramphenicol and the tetracyclines is founded. The evidence in this volume on which clinical practice is based will help to make general principles clearer. Methods of treatment are given in detail. The importance of accurate diagnosis is stressed.

Bergey's Manual of Determinative Bacteriology. 7th ed. By ROBERT S. BREED, E. G. D. MURRAY, NATHAN R. SMITH, *et al.* The Williams and Wilkins Company, Baltimore, 1957. xviii + 1,094 pp. 15 x 23 cm. Price $15.

The format of this edition differs but little from that of the sixth edition. Many changes in the content, however, as the result of a thorough-going revision are apparent. Much historical material in the sixth and earlier editions of the Manual has been excluded. The new edition represents the coordinated results of the work of 100 contributors.

Methods of Biochemical Analysis. Vol. V. Edited by DAVID GLICK. Interscience Publishers, Inc., New York, 1957. ix + 502 pp. 15 x 23 cm. Price $9.50.

This book is designed to present chemical, physical microbiological, and if necessary, animal assays; as well as basic techniques and instrumentation for the determination of enzymes, vitamins, hormones, lipids, carbohydrates, proteins and their products, minerals, antimetabolites, etc.

Biochemistry and Human Metabolism. 3rd ed. By BURNHAM S. WALKER, WILLIAM C. BOYD, and ISAAC ASIMOV. The Williams and Wilkins Company, Baltimore, 1957. vii + 937 pp. 15 x 23 cm. Price $12.

The main effort in preparing this edition has been to keep the text as nearly as possible abreast of the field indicated by the title. Parts of the text have been rewritten and re-arranged and much new material has been introduced.

Catalysis. Vol. V. Edited by PAUL H. EMMET. Rheinhold Publishing Corporation, New York, 1957. vi + 542 pp. 15 x 23 cm. Price $15.

This fifth volume includes reactions of carbon monoxide, the oxo reaction, the direct catalytic synthesis of higher alcohols from carbon monoxide and hydrogen, the catalytic hydrogenation of aromatic compounds, hydrogen isotopes in the study of hydrogenation and exchange, and the hydrodesulfurization of liquid petroleum fractions. Subsequent volumes will deal with halogenation, isomerization, alkylation, polymerization, cracking, decomposition, and miscellaneous reactions.

The Chronically Ill. By JOSEPH FOX. The Philosophical Library, Inc., New York, 1957. xix + 229 pp. 13.5 x 21.5 cm. Price $3.95.

The material contained herein is of special interest to the physician, the social worker, the hospital administrator, and to labor and management. This book is a sociological approach to the problem of conquering the ravages of chronic diseases.

Microtechniques of Clinical Chemistry for the Routine Laboratory. By SAMUEL NATELSON. Charles C Thomas, Publisher, Springfield, Ill., 1957. xv + 484 pp. 15 x 23 cm. Price $11.

This book is written for investigators interested in following the effect of disease or drugs on the human organism or small laboratory animals. It is also of value to those faced with analysis of inorganic and organic constituents present in small amounts in all fields of chemistry. The basic principles used in microanalysis and the equipment needed for this purpose are discussed.

Complexometric Titrations. By GEROLD SCHWARZENBACH. Translated and revised in collaboration with the author by Harry Irving. Interscience Publishers, Inc., New York, 1957. xviii + 132 pp. 14 x 22 cm. Price $3.75.

The author has presented a clear and full account of the basic theory in such a way that analysts may be able to plan new determinations and to understand the reasons for each step in the detailed procedures. The book contains a full account of the theory and practice of metal-indicators. The preparation of standard solutions of complexones and the necessary buffers and indicators are described. Full detailed accounts of specific determinations are given.

Digitalis. Compiled and edited by E. GREY DIMOND. Charles C Thomas, Publisher, Springfield, Ill., 1957. xiv + 255 pp. 15 x 23 cm. Price $7.

Collected in this book are the present day concepts of the use of digitalis, up-to-date ideas of dosage, treatment of intoxication, and preparations of choice.

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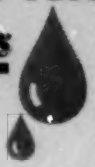
for tableting **or liquids** 

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